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STUDIES RELATING TO THE IMMUNOLOGY OF BOVINE INFECTIOUS ABORTION [1]

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INTRODUCTION

Artificial immunization as a means of combating infectious diseases of the domestic animals is a subject that during recent years has received no little amount of consideration. Numerous diseases which formerly exacted heavy losses are now being more or less successfully suppressed through the utilization of immunizing substances. As a result of the progress made along these lines, persons interested in disease-control work have naturally been much encouraged and led to entertain hope that such methods of combating disease may in the course of time be greatly extended.

REVIEW OF PREVIOUS WORK

Investigators for a number of years have been interested in determining whether bovine infectious abortion may be advantageously attacked through the agency of biological preparations. The subject has been of especial interest in connection with this disease because of the fact that the control of *Bacterium abortus* exposure in infected herds is extremely difficult and because the total elimination of the infection from such herds frequently is accomplished only at a financial sacrifice which renders such an attempt impracticable.

Efforts to evolve and evaluate biological methods of control have been prompted to a great degree by the observation that in recently infected herds the disease generally spreads rapidly and for a period causes excessive losses which, in the event that animals from other herds are excluded, show a tendency to gradually subside, or in the course of time even largely to disappear. It has seemed reasonable to ascribe the improvement in conditions, in part at least, to the development of a naturally acquired immunity and to infer that a resistance of similar character should be possible of development in an artificial manner.

Bang [2] in his early writings relative to the isolation of the abortion microorganism called attention to the possibilities of artificial immunization, and in a later report [3] recorded the results of his experiments in which both living and killed cultures of *Bacterium abortus* were employed. A practice was made of injecting nonpregnant animals either subcutaneously or intravenously a number of times and, following conception, subjecting them to a severe type of *Bacterium abortus* exposure. While many of his treated animals failed to withstand the degree of exposure to which they were subjected, the results obtained were nevertheless encouraging, particularly those derived from the subcutaneous administrations of living cultures.

[1] Received for publication Mar. 4, 1924.

[2] BANG, B.—DIE ÆTIOLOGIE DES SEUCHENHAFTEN ("INFECTIÖSEN") VERWERFENS. Ztschr. Thiermed. 1: 241-278, illus. 1897.

[3] BANG, B.—INFECTIOUS ABORTION IN CATTLE. Jour. Compar. Path. and Ther. 19: 191-202. 1906.

McFadyean and Stockman [4] in their early investigations of the disease also gave the matter of artificial immunization attention and described in detail experimental work dealing with two virgin heifers that received subcutaneously single massive doses of *Bacterium abortus* cultures. These animals were later bred and during their gestation periods received enormous amounts of material containing abortion bacteria. When destroyed in an advanced stage of pregnancy failure was experienced in detecting pathological changes in their uteri or in isolating therefrom the abortion organism. Such findings led these workers to conclude that the treatment practiced preceding conception conferred an active immunity.

Stockman [5] in discussing infection abortion at a later date gave further consideration to artificial immunization as a means of preventing abortion losses. He described results that were obtained in infected herds where single massive doses of living cultures or suspensions of *Bacterium abortus* were administered to nonpregnant animals and where heavy suspensions of killed abortion bacteria were administered to the pregnant animals at monthly intervals up to the sixth month of pregnancy. Instead of making use of chemical agents for killing the organisms, as was practiced by Bang in his preparation of bacterins, the suspensions were subjected to a temperature of 65° C. for one-half hour. Those animals receiving the viable organisms were not bred for a period of at least two months following the injections. Badly infected herds are mentioned as having been selected for experimentation. An estimate of the value of the immunizing procedure was arrived at by comparing the number of abortions that subsequently occurred in the treated groups with those that occurred in control animals in the same herds. With this method of evaluation the results derived from the employment of living abortion bacteria appeared to be highly encouraging, for whereas 23.4 per cent of the 432 controls aborted, the abortion rate in the 493 treated subjects was but 6.5 per cent. Inasmuch as 21 per cent of 110 animals that received repeated injections of killed organisms aborted, it was concluded that the benefits derived from the use of the killed organisms was not sufficiently marked to compensate for the labor involved in the making of the numerous injections.

Investigators in this and foreign countries, other than those previously mentioned, who have contributed data bearing on the subject of artificial immunization include the following: Giltner, Hallman, Williams, Hadley, Huddleson, Barnes, Hagan, Klimmer, Zwick and his coworkers, and others.

The results of immunizing experiments that have been reported from time to time have for the most part been disappointing where either killed abortion bacteria or serum with a high agglutinating titer for abortion bacteria have been utilized as immunizing agents. On the other hand, when viable abortion bacteria have been employed in connection with nonpregnant subjects, the resistance of the animals to the pathogenic action of *Bacterium abortus* has subsequently appeared to be materially increased.

THE PRESENT EXPERIMENTS

The experimental work recorded in this paper, which was inaugurated during the latter part of 1917, was prompted largely by the encouraging results previously mentioned as having been reported by Stockman in connection with the extensive immunizing work carried out in England. While the principal object

[4] McFadyean, J., and Stockman, S.—Report of the departmental committee appointed by the Board of Agriculture and Fisheries to inquire into epizootic abortion. Appendix to Part I. 43 p. London. 1909.

[5] Stockman, S.—Epizootic abortion. Internat. Vet. Cong. Rpt. 10 (2). 343-354. 1915.

in view in undertaking the work was that of procuring additional data with reference to the possibilities of immunization, it was also planned to give some consideration to the length of time that animals treated with living abortion organisms carry the infection in their bodies as a result of subcutaneous inoculations. That many noninfected animals might thereby be made carriers or disseminators of the disease seemed possible, in view of the fact that a rather high percentage of cows which acquire the disease under natural conditions have been shown to harbor the infection in their udders for long periods, and frequently eliminate the bacteria from their genital tracts at subsequent parturitions irrespective of whether they expel dead or seemingly normal calves. It was proposed in the immunizing experiments to employ both living and killed *Bacterium abortus* organisms, although the value of the latter in rendering animals resistant to infection had been seriously questioned by different investigators.

METHODS OF PROCEDURE

Two general methods of procedure were followed in applying the immunizing tests. In one of these use was made of infected herds where a portion of the animals were subjected to immunizing treatments and the remainder employed as controls. An attractive feature of this method was that it made available for experimentation a large number of animals. Bacteriological studies of aborting cows could, however, seldom be made, in consequence of which the interpretation of results was rendered somewhat complex and confusing. Impossibility of controlling *Bacterium abortus* exposure of individual animals under herd conditions, moreover, introduced another element of uncertainty.

In the second method of procedure the number of animals used was limited, but it was possible to give more attention to their selection and handling than could be done under herd conditions where experimental results were secondary. Care was exercised in the procuring of stock which was susceptible to the disease. Serological tests were conducted at frequent intervals, and cultural and guinea-pig inoculations of milk and uterine materials were made at such times as were considered advantageous or instructive. The results that were thereby obtained are regarded as being of greater interest and significance than those derived from the experiments carried out under herd conditions, and will therefore be discussed somewhat more in detail.

EXPERIMENTS WITH SELECTED STOCK

The stock referred to, consisting of 66 females and 15 bulls ranging from 1 to 3 years of age, was obtained from an isolated mountainous section of Tennessee into which, so far as could be determined, infectious abortion had not been introduced. Approximately 20 per cent of the heifers had been bred when purchased. Upon subjecting the entire shipment to the complement-fixation and agglutination tests negative reactions were obtained in all cases; that is, no fixation of complement and no agglutination of a *Bacterium abortus* suspension was produced by 0.04 cc. or lesser amounts of blood serum from any animal. Freedom from *Bacterium abortus* infection was further evidenced by the fact that no symptoms of abortion disease were manifested by any of the pregnant heifers that were permitted to complete their gestation periods before being used for experimental purposes.

Of the shipment described, 18 heifers and 5 cows were used in the experiment. Eight heifers and 3 cows received subcutaneous administrations of abortion vaccine [6] when nonpregnant, 4 heifers received abortion bacterin, and 6 heifers and 2 cows were used as controls.

[6] The term "vaccine" as used in this paper has reference to a suspension of living *Bacterium abortus*, while "bacterin" is used to indicate killed suspensions of the organism.

A 20-cc. dose has been used in practically all immunizing work that has been undertaken with living abortion bacteria, whether under herd conditions, where natural *Bacterium abortus* exposure has prevailed, or in connection with animals that have been utilized in a purely experimental capacity. This quantity of vaccine represents one-eighth of the growth of abortion organisms developing on approximately 15 square inches of 3 per cent glycerin-agar after seven days' incubation at a temperature of 37.5° C. When dilutions of the vaccine have been plated the number of colonies that have developed has indicated that the product contained approximately one billion organisms per cubic centimeter. Fifteen to twenty Blake bottles of medium have usually been sown at one time with suspensions of as many different strains, and a practice has been made of using only such strains as have been artificially cultivated for at least a year. After seven days' incubation the growth in each bottle was suspended in 160 cc. physiological salt solution. While there has been some variation in the amount of growth that has developed in the different bottles, vaccine of fairly uniform density has been obtained by combining the suspensions of each lot. The quantity of vaccine administered to each animal has been considerably less than that mentioned by Stockman, who refers to the organisms obtained from a similar area of medium as constituting a dose for but one or two subjects.

The vaccinated animals were mated with a bull that was used exclusively for serving those so treated and which gave negative complement-fixation and agglutination reactions throughout the experiment. The bull that was mated with the bacterin-treated group, as well as those to which the controls were bred, were protected from abortion exposure, and gave negative reactions to the serological tests at all times.

The animals receiving bacterin were treated as follows: Two heifers, No. 423 and 404, received subcutaneous injections two and four times, respectively, before being bred, and five times each before being subjected to *Bacterium abortus* exposure. The injections were continued monthly in doses of 20 cc. The suspensions were identical in density with the vaccine described above, but were placed in a water bath and maintained at a temperature of 65° C. for one-half hour. One heifer, No. 464, was treated bimonthly with half the dose of killed organisms and received two injections previously and seven subsequently to exposure. The fourth heifer, No. 401, received bimonthly injections of 20 cc., two of which were given previously to exposure but subsequently to breeding date. This animal received seven treatments in all. Individual stalls were provided for the treated as well as the control animals during their gestation periods, and all the animals were handled in such a manner as to reduce to a minimum the possibility of contact with *Bacterium abortus* infection other than that intentionally furnished.

The method of exposure to *Bacterium abortus* was as follows: Varying quantities, from 5 to 60 cc., of stomach contents of aborted fetuses, from which *Bacterium abortus* and no other microorganisms could be isolated, were administered by the mouth. The material was diluted with from 50 to 100 cc. of physiological salt solution. One feeding was practiced for the most part, although in a few instances it was considered desirable to administer the material a second time. While numerous lots of stomach contents were required for the exposure of the 23 animals utilized, by reason of the fact that the breeding dates extended over a period of considerable length, efforts were made to breed the animals at such times as would permit of the exposure of those treated and of the controls being as nearly uniform in character as possible.

RESISTANCE OF TREATED AND CONTROL ANIMALS

During the first gestation period 10 of the 11 vaccinated animals produced living calves. One abortion occurred in this group, heifer No. 447, from the fetus of which *Bacterium abortus* was isolated. Another animal, cow No. 410, produced a living calf at the expiration of a 229-day gestation period. When she was slaughtered ten days later *Bacterium abortus* was isolated from her uterus. Failure was experienced in demonstrating the presence of *Bacterium abortus* in the placentas or uterine fluids of any of the other 9 animals of this group at time of parturition, although cultural efforts and guinea-pig inoculations were practiced in all cases.

In the bacterin-treated group 2 heifers, No. 423 and 401, produced living calves at the expiration of gestation periods of 282 and 273 days, respectively. Failure was experienced in isolating *Bacterium abortus* from the placentas or uterine fluids of these 2 animals at time of parturition. Bacterin-treated heifers 404 and 464 expelled dead calves that were shown to harbor *Bacterium abortus* infection.

Any comparison of the amount of exposure in these cases with that sustained by animals in infected herds under natural conditions is more or less conjectural, but that it was severe is evidenced by the fact that 7 of the 8 controls subsequently aborted, and *Bacterium abortus* was isolated from the fetuses, placentas, or uterine fluids of all 8 subjects.

Table I shows the dates of vaccination, the intervals between treatment and breeding dates, the time that elapsed between conception and exposure, the length of the gestation periods, and the final results obtained.

TABLE I.—*Immunization results in animals subjected to artificial exposure to Bacterium abortus*

TREATED WITH ABORTION VACCINE

Experimental animal	Date of treatment	Date of breeding	Date of exposure	Exposure material (fetal-stomach contents)		Gestation period	Outcome of pregnancy	Results of uterine examination for *Bact. abortus*
				Case No.	Amount			
					Cc.	*Days*		
Heifer 402	Mar. 2, 1917	Apr. 27, 1917	Nov. 2, 1917	42	5	276	Living calf	Negative.
		Aug. 29, 1918	Nov. 13, 1918	74	50	279	do	Do.
		Oct. 3, 1919	Mar. 18, 1920	110	20	280	do	Do.
			May 10, 1920	126	22			
Heifer 409	Mar. 2, 1917	Apr. 3, 1917	June 6, 1917	21	50	282	do	Do.
	Mar. 23, 1917	July 24, 1918	Aug. 20, 1918	70	25	280	do	Do.
		Oct. 24, 1919	Mar. 18, 1920	110	20	284	do	Do.
			May 10, 1920	126	20			
Heifer 417	Feb. 1, 1917	May 25, 1917	Dec. 13, 1917	84	30	272	do	Do.
		July 12, 1918	Aug. 20, 1918	70	30	267	do	Do.
		Oct. 24, 1919	Mar. 18, 1920	110	20	279	do	Do.
			May 10, 1920	126	22			
Heifer 418	Mar. 2, 1917	July 25, 1918	Aug. 20, 1918	70	25	284	do	Do.
	Mar. 23, 1917	Oct. 8, 1919	Mar. 18, 1920	110	20	277	do	Do.
			May 10, 1920	126	22			
Heifer 419	Mar. 30, 1918	Dec. 30, 1918	Feb. 24, 1919	80	60	281	do	Do.
			Mar. 19, 1919	88	25			
		Apr. 21, 1920	May 10, 1920	126	22	281	do	Do.
Heifer 427	Mar. 2, 1917	Apr. 6, 1917	June 8, 1917	21	50	282	do	Do.
		July 17, 1918	Aug. 20, 1918	70	25	278	do	Do.
		Oct. 3, 1919	Mar. 18, 1920	110	20	282	do	Do.
			May 10, 1920	126	22			
Heifer 462	Jan. 16, 1919	Mar. 12, 1919	May 24, 1919	92	50	285	do	Do.
		Apr. 26, 1920	May 10, 1920	126	22	284	do	Do.
Heifer 447	Mar. 30, 1918	July 1, 1918	Nov. 13, 1918	74	30	289	Abortion	Positive.
Cow 408	Feb. 24, 1919	Apr. 12, 1919	May 24, 1919	92	50	283	Living calf	Negative.
Cow 410	Mar. 2, 1920	Apr. 22, 1920	May 10, 1920	126	22	229	Weak calf	Positive.
Cow 416	Mar. 2, 1920	May 5, 1920	May 10, 1920	126	22	290	Living calf	Negative.

TABLE I.—*Immunization results in animals subjected to artificial exposure to Bacterium abortus*—Continued

TREATED WITH ABORTION BACTERIN

Experimental animal	Date of treatment	Date of breeding	Date of exposure	Exposure material (fetal stomach contents)		Gestation period	Outcome of pregnancy	Results of uterine examination for *Bact. abortus*
				Case No.	Amount			
					Cc.	*Days*		
Heifer 423	Feb. 1, 1917 Mar. 2, 1917 Apr. 2, 1917 May 2, 1917 June 7, 1917 July 2, 1917 Aug. 4, 1917 Sept. 4, 1917 Oct. 5, 1917 Nov. 3, 1917 Dec. 3, 1917	Mar. 23, 1917	June 8, 1917	21	50	282	Living calf	Negative.
		June 5, 1918	Aug. 20, 1918	70	25	231	Abortion	Do.
Heifer 401	Dec. 7, 1917 Jan. 2, 1918 Jan. 22, 1918 Feb. 2, 1918 Mar. 4, 1918 Mar. 25, 1918 Apr. 15, 1918	Aug. 8, 1917	Jan. 5, 1918	54	30	273	Living calf	Do.
		Sept. 23, 1918						
		Oct. 14, 1918	Nov. 3, 1918	74	20	278	do	Do.
Heifer 404	Feb. 1, 1917 Mar. 1, 1917 Apr. 2, 1917 May 2, 1917 June 2, 1917 July 2, 1917 Aug. 4, 1917 Sept. 4, 1917 Oct. 5, 1917	May 24, 1917	June 7, 1917	21	50	162	Abortion	Positive.
Heifer 464	Oct. 5, 1917 Oct. 20, 1917 Nov. 2, 1917 Nov 16, 1917 Dec. 3, 1917 Dec. 18, 1917 Jan. 2, 1918 Jan. 31, 1918 Feb. 14, 1918	June 25, 1917	Nov. 2, 1917	42	5	238	do	Do.

CONTROLS

Heifer 446		May 4, 1917	June 8, 1917	21	50	182	Abortion	Positive.
Heifer 422		May 4, 1917	June 8, 1917	21	50	177	do	Do.
Heifer 460		June 16, 1917	Nov. 2, 1917	42	5	265	do	Do.
Heifer 450		Aug. 16, 1917	Dec. 13, 1917	54	30	241	do	Do.
Heifer 413		May 9, 1917	June 8, 1917	21	50	206	do	Do.
Heifer 430		Jan. 7, 1919	Feb. 24, 1919 Mar. 19, 1919	80 88	20 25	223	do	Do.
Cow 470		Feb. 24, 1919	May 24, 1919	92	50	266	Living calf	Do.
Cow 453		Dec. 18, 1919	May 10, 1920	126	22	186	Abortion	Do.

The source of the fetal-stomach contents used for exposure purposes is indicated in Table I in order that animals receiving similar exposure may be readily identified. The fetal material was transferred to sterile flasks at the times the fetuses were cultured and was held at refrigerator temperature until cultural results were ascertained.

It may be observed by reference to Table I that seven vaccinated animals were carried through a second period of pregnancy without further administration of vaccine and that living calves were produced in all cases. Failure was furthermore experienced in demonstrating the presence of *Bacterium abortus* in the placentas or uterine fluids of these animals at time of parturition. Four of the

animals were carried through a third period and gave further evidence of immunity, after a third exposure, by producing living calves and by the absence of uterine infection as indicated by the cultural and guinea-pig inoculation work that was carried out.

It may also be observed by reference to Table I that but 3 cows were subjected to the vaccination procedure, the remaining 8 being heifers. The udders of these 3 were shown to harbor *Bacterium abortus* at time of parturition. The presence of the infection in 2 of these subjects, 408 and 416, was not demonstrated previously to the ingestion exposure, in consequence of which it can not be assumed that the vaccine treatment was responsible for the condition. *Bacterium abortus* was, however, isolated from the milk of cow No. 410 forty-four days subsequently to vaccination and previously to date of ingestion exposure. Although this animal produced a living calf at the expiration of a 229-day gestation period, the vaccine treatment is looked upon as probably having been responsible for both the premature expulsion of the fetus and the presence of *Bacterium abortus*, which was shown to exist in her uterus at time of parturition.

Animals No. 423 and 401, which gave evidence of having derived some protection from treatment with bacterin, were carried through a second pregnant period without further efforts at immunization. Animal No. 423 had received 11 and animal No. 401, 7 injections of killed abortion bacteria previously to first pregnancy. Cow No. 423 aborted at the expiration of 231 days, but failure was experienced in demonstrating the presence of *Bacterium abortus* in either the fetus or the other products of the abortion. *Bacterium abortus* infection of the udder was demonstrated in cow No. 401 during and at termination of this pregnancy, although its presence in the uterus at time of parturition could not be established.

OBSERVATIONS WITH REGARD TO SEROLOGICAL TESTS

Bimonthly applications of the serological tests to the blood serum of the animals used in the experiment proved to be of interest. It could reasonably be inferred from the character of the reactions obtained that in none of the vaccinated heifers did *Bacterium abortus* infection persist for any great length of time following treatment. In these animals it was observed that a decrease in agglutinins and complement-fixing bodies began soon after vaccination and gradually continued until their blood serum exhibited slight or no agglutinating or complement-fixing properties. Cow No. 410 was the only vaccinated animal that continued to react in a pronounced manner to the tests during the entire gestation period, and, as previously stated, she harbored the infection in her udder 44 days subsequently to the treatment.

The charts of the individual animals (Table V, p. 621) show some degree of variation in the results of the agglutination tests that were obtained at the different testing dates, as well as between the agglutination and complement-fixation reactions, particularly in those animals that received injections of abortion vaccine. The blood serum of these animals usually possessed some agglutinating properties in the quantities employed, when complement-fixing bodies could no longer be demonstrated. The results obtained made it appear evident that slight reactions to the agglutination test may persist for long periods after infection of the animal has apparently been overcome. It is somewhat questionable, however, whether the agglutinin content of the sera from the treated animals varied from time to time to the extent indicated by the results recorded. Slight variations in density of the test fluid employed, a difference in the age of the suspensions, a certain degree of variation in the length of the period that the tests were incubated, and the personal factor involved in the reading of the tests were features that singly or in combination may have been largely responsible for such variations in results as the charts indicate.

The quantities of serum that were used in making the agglutination tests were 0.04, 0.02, 0.01, 0.005, 0.002, and 0.001 cc. To these quantities were added 1 cc. of a suspension of abortion organisms similar in density, as determined by the use of a Kober nephelometer, to a suspension of barium sulphate prepared

by combining 1 cc. of a 1-to-100 solution of barium chlorid with 99 cc. of a 1-to-100 solution of sulphuric acid. A practice was made of reading the tests after they had been incubated at 37° C. for a period of from 36 to 48 hours. The degree of agglutination has been indicated (in Table V) by the characters +, P, S, and —. The + sign has been used to indicate complete clumping of the bacteria, P a partial clumping, S a slight trace of agglutination, and the minus sign to indicate no evidence of clumping.

In addition to the bimonthly agglutination and complement-fixation results indicated in the charts of the individual animals, the results of cultural and inoculation tests of milk, placentas, fetuses, uterine fluids, etc., are also recorded.

OBSERVATIONS RELATIVE TO IMMUNITY IN ANIMALS WHICH HAD PREVIOUSLY ABORTED

At the time this experimental work was undertaken there appeared to be a difference of opinion as to the theory of living organism vaccination. The use of massive doses of *Bacterium abortus* created the impression that saturation of the subject with abortion bacteria was the object desired. Under these conditions the prevention of abortion losses was attributed more to the development of a tolerance to abortion bacteria than to resistance to their invasion. This conception appeared to be justified to some degree in view of the fact that little distinction was made between infected and noninfected animals as proper subjects for immunization. In other words, the tendency was to assume that the noninfected animal could be rendered resistant to the infection by the subcutaneous administration of massive doses of the organisms, and that the *Bacterium abortus* udder-infected animal likewise derived resistance of a similar character by contributions to the infection borne at the time of treatment and possibly for months or years previously. Whether or not injections of biological products have a tendency to overcome infection of the udder with abortion bacteria is a problem that has received very little attention. In the absence of evidence to support such a contention it is difficult to conceive logically how the same beneficial results can be reasonably anticipated from the treatment of both types of animals.

Studies that were made of 12 cows, all of which had previously aborted, and from the fetuses, placentas, or uterine fluids of which *Bacterium abortus* had been isolated, furnished information that has some bearing on this matter. All of these animals were eventually successfully bred. Their milk was then tested for the presence or absence of *Bacterium abortus* by guinea-pig inoculations. It was determined that the milk produced by one-third of the number contained abortion bacteria. The milk from eight of the group failed to produce abortion disease in any of the pigs that were inoculated.

Subsequently to collecting the milk samples, 11 of the 12 cows received by the mouth variable amounts of fetal-stomach contents containing *Bacterium abortus*. One cow received one administration, 4 received the material on two occasions, and 6 on three different dates. Cultural tests which were made of the exposure material in all cases indicated the presence of viable abortion bacteria in an uncontaminated condition. Ten living calves were produced by this group. Failure was experienced at time of parturition in isolating *Bacterium abortus* from the placentas or uterine fluids of eight of the number by cultural means or guinea-pig inoculations. The animals having uteri free from *Bacterium abortus* at time of parturition, as indicated by the above-mentioned tests, were those that from the earlier milk tests gave evidence of having overcome the infection in their bodies.

Bacterium abortus was isolated from the fetuses, placentas, or uterine fluids of the remaining four cows, despite the fact that animal No. 413 was not subjected to ingestion exposure during the present pregnant period and two of the number (413 and 460) aborted. All four were shown to have been carriers of *Bacterium abortus* infection in their udders during the early months of pregnancy. (See Table II.)

TABLE II.—*Exposure tests of aborting cows to determine the degree of immunity resulting from Bacterium abortus infection*

TABLE II.—*Exposure tests of aborting cows to determine the degree of immunity resulting from Bacterium abortus infection*

No. of animal	Date of previous abortion	Date of breeding	Date milk was obtained for inoculation purposes	Results of milk examinations for *Bact. abortus*	Date of exposure	Exposure material (fetal-stomach contents)		Gestation period	Outcome of pregnancy	Results of uterine examination for *Bact. abortus*
						Case No.	Amount			
							Cc.	*Days*		
422	Oct. 28, 1917	Feb. 12, 1918 Mar. 22, 1918 May 21, 1918 June 29, 1918 July 17, 1918	Aug. 8, 1918	Negative	Nov. 13, 1918	74	50	281	Living calf	Negative.
407	Nov. 2, 1917	Mar. 13, 1918 Feb. 20, 1918	Aug. 14, 1918	do	Aug. 20, 1918 Nov. 19, 1918	70 74	50 5	288	do	Do.
454	Nov. 19, 1917	Feb. 20, 1918 Mar. 26, 1918 May 21, 1918 June 2, 1918	Aug. 14, 1918	do	Aug. 20, 1918 Nov. 19, 1918	70 74	50 5	297	do	Do.
473	Feb. 18, 1918	Apr. 13, 1918	Aug. 14, 1918	do	Aug. 20, 1918 Nov. 19, 1918	70 74	50 5	287	do	Do.
316	Nov. 18, 1917	Nov. 18, 1918	Aug. 9, 1918	do	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919	80 88 92	60 25 50	285	do	Do.
405	May 13, 1917	Jan. 17, 1919	Feb. 24, 1919	do	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919	80 88 92	60 25 50	280	do	Do.
460	Apr. 14, 1918	Oct. 10, 1918	Feb. 24, 1919	do	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919	80 88 92	60 25 50	282	do	Do.
317	Dec. 18, 1916	Oct. 21, 1918	Aug. 9, 1918	do	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919	80 88 92	60 25 50	282	do	Do.
446	Nov. 2, 1917	Mar. 9, 1918	Aug. 8, 1918	Positive	Aug. 20, 1918 Nov. 19, 1918	70 74	50 5	268	do	Positive.
456	Sept. 3, 1918	Dec. 18, 1918	Feb. 24, 1919	do	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919	70 88 92	60 25 50	285	do	Do.
460	Mar. 18, 1918	June 5, 1918 Sept. 9, 1918 Dec. 21, 1918	Feb. 24, 1919	do	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919	80 88 92	60 25 50	167	Abortion	Do.
413	Dec. 1, 1917	Mar. 15, 1918 Apr. 15, 1918 May 13, 1918 June 26, 1918 July 26, 1918 Aug. 9, 1918 Aug. 28, 1918 Nov. 2, 1918	May 19, 1919	do				223	do	Do.

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The manner in which the pregnancies of these 12 animals with an abortion history terminated is regarded as being of particular interest, since it not only indicates that animals may derive a marked degree of immunity as a result of having sustained an attack of the disease, but suggests as well that it may be essential that centers of *Bacterium abortus* infection within the animal shall have ceased to exist before such immunity may readily be demonstrated.

While the fetus, placenta, and uterine exudate expelled by cow No. 413 during 1917 were heavily saturated with abortion bacteria, and from the numerous milk examinations made it could reasonably be concluded that her udder carried the infection continuously until aborting the second time, during June, 1919, little benefit in the way of tolerance to the organism appears to have been acquired. When the manner in which the pregnancy of this animal as well as of the 3 other udder-infected cows used in the exposure experiment terminated is compared with the 8 that gave evidence of having overcome the infection in their bodies, it seems reasonable to infer that the pregnant periods of carriers may well be regarded as speculative so long as the infection persists, and that its total elimination may be necessary before a dependable and true immunity is established. This feature is presumably responsible to a great degree for the differing views held as to whether immunity is derived from an attack of the disease.

DISCUSSION OF IMMUNIZATION RESULTS

Considerable encouragement was derived from the results obtained with living abortion organisms under the experimental conditions described. It was gratifying to observe that although marked serological reactions were produced in susceptible heifers by the injections, they became much reduced in intensity or disappeared in a comparatively brief period. This feature was regarded as being of especial interest, inasmuch as what has heretofore appeared to be a logical argument against the use of living organisms has been that heifers so treated may thereafter be rendered spreaders of the disease in herds into which they may be subsequently introduced. The entire absence of sterility troubles and freedom from calving complications in this group of vaccinated subjects constituted further grounds for optimism relative to the procedure, and the simplicity of the immunizing method made it appear especially attractive.

It was only when experimental animals with functioning udders were vaccinated that a certain amount of danger of producing carriers of the disease by the treatment, and of possible defeat of the object desired, was definitely determined. A limited amount of experimental work has been directed toward determining with what frequency the organisms localize in the udders of vaccinated nonpregnant cows. Despite the fact that the percentage of such cases was relatively high in the few mature animals employed in the original experimental work, additional data pertaining to this feature have failed to indicate that as much cause for alarm exists as was suggested by the earlier experiments.

Twenty cows from a herd regarded as being free from infectious abortion were introduced into a herd in which severe abortion losses were being experienced and where 75 per cent of the animals gave positive reactions to the agglutination test for the disease. These 20 animals gave negative results to the agglutination test for infectious abortion at the time of purchase. On November 2, 1921, which was shortly after the introduction of the group, they were injected subcutaneously with 20 cc. of abortion vaccine. On March 6, 1922, samples of blood and milk were taken from those treated and were subjected to the agglutination test. In those cases where 0.005 cc. or lesser amounts of the blood serum caused any agglutination, or where agglutination of the milk occurred even in 0.04 cc. or lesser amounts, guinea-pig inoculations were made with samples of the milk sediment. While 9 groups of guinea pigs consisting of 3 each were injected with sediment

from the milk samples, in no instance could the presence of abortion disease be demonstrated in the guinea pigs when they were destroyed, April 12, 1922, although serological and cultural tests were conducted.

Another group of 11 cows that gave negative results to the agglutination test was vaccinated November 17, 1921. Samples of blood and milk were obtained from these animals June 24, 1922, and tested by the agglutination method. In no case did any of the milk samples show agglutination with 0.04 cc. or lesser amounts. The blood serum of 3 animals showed slight clumping in 0.01 cc. quantities. The serum reactions of the remaining 8 were either negative or showed a titer of less than 0.01 cc. Two guinea pigs were injected with 4 cc. of milk from each cow of this group. When destroyed, two months later, all the pigs yielded negative serological and cultural results for *Bacterium abortus*.

When consideration is given to the results obtained with the 23 animals that were used in a purely experimental capacity, it seems important to bear in mind that so far as could be determined they were free from *Bacterium abortus* infection and susceptible to the disease, and, furthermore, that the vaccination procedure gave indication of being successful only when temporary infection of the animal was occasioned by the treatment. Our knowledge of immunity with reference to other diseases enables us to conceive how under these conditions the administration of abortion vaccine may constitute a reasonable and rational immunizing procedure.

Abortion bacterin failed to show highly encouraging results in this experiment. Although 2 of the 4 animals utilized successfully withstood *Bacterium abortus* exposure that caused controls to abort, it should be borne in mind that this product was used under what may be regarded as ideal conditions; that is, noninfected heifers were used to test the efficacy of the method, and repeated injections of generous amounts were made not only previously to exposure, but in the case of one aborter 4 times previously to conception. Indication that the immunity derived from the bacterin treatment was of but low degree and transient is evidenced by the fact that cow No. 401 acquired *Bacterium abortus* udder infection following her second ingestion exposure. Although failure was experienced in demonstrating that bacterin-treated animal 423 was infected with *Bacterium abortus* following her second exposure, the character of her serological reactions indicated that such may have been the case.

VACCINE TREATMENT UNDER HERD CONDITIONS

Abortion vaccine has been used in a number of infected herds with the object of acquiring further information as to the practical value of such a product in combating the malady. The interpretation of results under these conditions has not been wholly satisfactory, since, as has been previously mentioned, the matter of *Bacterium abortus* exposure could be controlled only in a measure, and because it has seldom been possible to conduct the necessary bacteriological work during such investigations to permit of differentiating between abortions of *Bacterium abortus* causation and those induced by other factors. Brief consideration, however, will be given to one herd consisting of more than 1,500 head of dairy stock, in which the disease had been prevalent for a number of years, and in which the vaccine was used for the treatment of the nonpregnant animals.

As animals in this herd became available for treatment blood samples were obtained for the application of the agglutination test. Available animals included open cows that had freshened at least two weeks previously and heifers that were within two or three months of breeding age. Two-thirds of the subjects from which blood samples were taken, which group included positive and atypical reactors as well as negative animals, thereupon received a subcutaneous injection of 20 cc. of vaccine, the remaining one-third being employed as controls.

The results derived from the utilization of 1,141 animals under herd conditions are indicated in Tables III and IV.

TABLE III.—*Results of vaccine treatment in a Bacterium abortus infected dairy herd*

VACCINATED ANIMALS

Original No.	Sold or died	First period of gestation: Conceived No.	Conceived Per cent	Sterile No.	Sterile Per cent	Produced living calves No.	Produced living calves Per cent	Aborted No.	Aborted Per cent	Second period of gestation: Sold or died subsequently to first pregnancy	Calved or aborted a second time	Produced living calves No.	Produced living calves Per cent	Aborted No.	Aborted Per cent	Number of animals with incomplete record of second gestation period [a]
772	91	617	90.6	64	9.4	536	86.9	81	13.1	128	311	279	89.7	32	10.3	175

CONTROLS

369	46	294	91.0	29	9.0	242	82.3	52	17.7	63	142	122	85.9	20	14.1	89

[a] Circumstances necessitated the discontinuance of this experiment before second gestation periods of a considerable number of animals terminated.

TABLE IV.—*Grouping of animals shown in Table III with regard to their agglutination reactions*

HEIFERS WHICH GAVE NEGATIVE AGGLUTINATION REACTIONS JUST PRIOR TO VACCINATION

Original No.	Sold or died	First period of gestation: Conceived No.	Conceived Per cent	Sterile No.	Sterile Per cent	Produced living calves No.	Produced living calves Per cent	Aborted No.	Aborted Per cent	Second period of gestation: Sold or died subsequently to first pregnancy	Calved or aborted a second time	Produced living calves No.	Produced living calves Per cent	Aborted No.	Aborted Per cent	Number of animals with incomplete record of second gestation period [a]
266	23	229	94.2	14	5.8	211	92.1	18	7.9	53	86	76	88.4	10	11.6	90

HEIFERS WHICH GAVE NEGATIVE AGGLUTINATION REACTIONS JUST PRIOR TO USE AS CONTROLS

130	17	110	97.3	3	2.7	95	86.4	15	13.6	27	39	32	82.0	7	18.0	44

COWS WHICH GAVE NEGATIVE AGGLUTINATION REACTIONS JUST PRIOR TO VACCINATION

292	42	234	93.6	16	6.4	210	89.7	24	10.3	50	144	131	91.0	13	9.0	49

COWS WHICH GAVE NEGATIVE AGGLUTINATION REACTIONS JUST PRIOR TO USE AS CONTROLS

128	23	97	92.4	8	7.6	84	86.6	13	13.4	17	60	55	91.7	5	8.3	20

COWS AND HEIFERS WHICH GAVE POSITIVE OR ATYPICAL AGGLUTINATION REACTIONS JUST PRIOR TO VACCINATION

214	26	154	82.0	34	18.0	115	74.7	39	25.3	25	81	72	88.9	9	11.1	43

COWS AND HEIFERS WHICH GAVE POSITIVE OR ATYPICAL AGGLUTINATION REACTIONS JUST PRIOR TO USE AS CONTROLS

111	6	87	82.9	18	17.1	63	72.4	24	27.6	19	43	35	81.4	8	18.6	25

[a] Circumstances necessitated the discontinuance of this experiment before second gestation periods of a considerable number of animals terminated.

In this herd 772 vaccinated animals are contrasted with 369 controls. During the first gestation period 13.1 per cent of those treated which conceived aborted, whereas 17.6 per cent of the successfully bred controls expelled dead calves. During the second pregnant period, when records were kept of 311 of the vaccinated subjects and 142 controls, 10.2 per cent of the treated animals aborted as against 14 per cent of the controls. The vaccinated animals received no immunizing treatment between the two gestation periods.

In Table IV these 1,141 animals are grouped in such a manner as to give the results of the treatment of heifers and cows that gave negative agglutination reactions at the time they were utilized and animals that gave positive and atypical reactions. Reactions were designated atypical when clumping of 1 cc. of a *Bacterium abortus* suspension was caused by 0.02 cc. or 0.04 cc. of blood serum but not by lesser quantities.

Analysis of the figures indicates that the negative heifers derived the greatest amount of benefit from the treatment. The treated heifers aborted at the rate of 7.9 per cent during the first period and 11.6 per cent during the second, whereas the rate of abortions in the controls was 13.6 per cent during the first period and 18 per cent during the second.

Treated negative cows aborted at a somewhat lower rate than the controls during the first period but at slightly higher rate during the second.

The abortion rate in the positive and atypical group was exceedingly high during the first period. The vaccination of this group gave indication of having caused very slight if any improvement in conditions.

It may be observed by reference to Tables III and IV that when the experimental work in the herd was discontinued sufficient time had not elapsed to permit of obtaining complete data on the second gestation periods, subsequent to vaccination, of all animals. The percentages of animals which aborted and of those which produced living calves during this pregnancy are based upon the performance only of those with a second complete breeding history subsequent to their experimental use.

The benefit derived from the use of the vaccine in this herd, even from the treatment of negative heifers, was somewhat less pronounced than was anticipated in view of the encouraging results obtained by the same method of immunization when negative heifers were subjected artificially to definite amounts of *Bacterium abortus* exposure following vaccination. It was suspected that in this herd factors other than *Bacterium abortus* infection might have been responsible in no small degree for the abortion losses sustained, whereupon blood samples for the application of the agglutination test were taken from 47 animals that aborted after being utilized in the experiment. Thirty of these were included in the vaccinated and 17 in the control group. The 30 aborters in the vaccinated group gave reactions to the agglutination test as follows: Seven positive, 1 atypical, and 22 negative. In the control group there were 10 positive and 7 negative.

The average length of period that intervened between the dates of aborting and the second test was 9½ months. Nineteen of the 47 aborters that were tested by the agglutination method in less than 4 months following the abortion date gave reactions as follows: Eight, or 42.1 per cent positive, and 11, or 57.8 per cent, negative. The remainder of the aborting group, which consisted of 28 animals that were tested between 4 and 23 months after aborting, gave reactions as follows: Ten, or 35.7 per cent, positive, and 18, or 64.2 per cent, negative. While it is possible that the results of the second tests might have differed to a certain extent had they been made in all cases at or near the time the abortions occurred, the percentage of aborters in the two groups, that is, those tested shortly following aborting and those after a more prolonged interval that gave

positive serological reactions, does not differ so greatly as to suggest that the results of the tests were greatly misleading.

When it is taken into consideration that the agglutination results obtained indicated that approximately 60 per cent of these abortions were induced by factors other than *Bacterium abortus* infection, the cause for failure on the part of the vaccine in this herd to yield results more in harmony with those obtained with animals that were subjected to artificial *Bacterium abortus* exposure becomes more readily apparent. These results emphasize the danger of drawing erroneous conclusions as to the value of immunizing agents in infectious abortion unless efforts be made to distinguish between abortions of *Bacterium abortus* and other types of causation.

By referring to the results obtained in the experimental group of 23 animals as indicated in Table I, it will be seen that the administration of fetal-stomach contents containing *Bacterium abortus* proved to be a very effective means of transmitting the disease to susceptible animals, as all the controls in the group, 8 in number, were thus infected. In exposure experiments by different investigators who have used *Bacterium abortus* cultures the percentage of positive results has frequently not been so great. It has therefore seemed reasonable to suspect either that artificial cultivation of the organism causes a considerable reduction in its virulence or that substances of the character of fetal-stomach contents, uterine exudates, etc., that contain the infection may act as a protective envelope for the microorganisms in the alimentary canal until invasion of the tissues occurs.

The amount of material administered to most of the susceptible control animals was presumably greatly in excess of the quantity necessary to cause their infection, as is evidenced by the fact that 5 cc., 22 cc., and 30 cc. resulted in a manner similar to a 60-cc. dosage.

Viscous uterine exudates heavily saturated with *Bacterium abortus* have given indication of possessing infective properties similar to that of *Bacterium abortus* infected fetal-stomach contents when administered by way of mouth, whereas milk in 500-cc. quantities from cows with *Bacterium abortus* infected udders has failed to infect when administered in a similar manner.

When it is taken into consideration that a single feeding of as small a quantity as 5 cc. of fetal-stomach contents proved to be sufficient to produce the disease in a susceptible heifer, then it is reasonably obvious why the malady is often rapidly disseminated in susceptible herds following exposure to infected subjects discharging *Bacterium abortus*.

The length of the period that intervened between dates of ingestion exposure of the susceptible control subjects and the dates when agglutinins or complement-fixing bodies could be demonstrated is of practical interest, since it gives some idea as to how much confidence should be placed in the serological tests for detecting cases of recent infection. This period in the 7 control animals that were tested at bimonthly intervals showed considerable variation. The tests, moreover, in some cases indicated the presence of specific agglutinins soon after their ingestion exposure and their partial disappearance for a period when their presence in increasing amount could again be demonstrated. Two heifers (No. 413 and 446) remained negative to the agglutination test for approximately 4 months following exposure, and heifer No. 460 for 3½ months. A 6 weeks' interval elapsed before serological evidence of infection was obtained in the case of heifer No. 450. Blood serum from animals No. 422, 453, and 470 showed agglutinating properties in less than a month following exposure. The average length of time required for the demonstration of agglutinins was a trifle more than 2 months. Somewhat more time was necessary for the detection of complement-fixing bodies than of agglutinins, a feature which has been observed in other diseases where both methods of diagnosis are used.

CONCLUSIONS

The data derived from the experimental procedure described seem to justify the following conclusions:

1. That it is possible to demonstrate in cattle an immunity to *Bacterium abortus* infection.

2. That such immunity may be demonstrated both in cows that have sustained an attack of the malady, but give evidence of having overcome the infection in their bodies, and in susceptible animals that have been subjected, previously to conception, to the subcutaneous administration of viable abortion bacteria.

3. That whatever immunity may be engendered by the treatment of animals with abortion bacterin, even when administered in large and frequent dosages both preceding and following conception, is limited and transitory in character.

4. That the vaccination of abortion-infected animals, as well as of those which have overcome the infection, is in all probability an illogical and valueless procedure.

5. That the vaccination of nonpregnant heifers near breeding age with suspensions made from strains of *Bacterium abortus* that have been under artificial cultivation for a year or longer appears seldom to result in the permanent infection of such animals.

6. That the possibility of causing permanent infection is greater when the vaccinated animals have functioning udders.

7. That susceptible heifers may occasionally remain negative to the serological tests for this disease for a period of at least four months after *Bacterium abortus* exposure of such severity as to result subsequently in abortions.

8. That through the ingestion of fetal-stomach material containing *Bacterium abortus* susceptible pregnant animals may readily become infected with the disease.

TABLE V.—*Detailed records of individual experimental animals* [a]

VACCINATED HEIFER NO. 402

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Feb. 28, 1917	−	−	−	−	−	−	−	−	−	−	−	Mar. 2, 1917, subcutaneous injection of 20 cc. abortion vaccine.
Mar. 7, 1917	+	−	−	−	−	−	−	−	−	−	−	
Mar. 21, 1917	+	+	+	+	P	P	+	+	+	S	−	
Apr. 5, 1917	+	+	+	+	S	−	+	+	+	−	−	
Apr. 21, 1917	+	+	+	+	−	−	+	+	+	−	−	Apr. 27, 1917, bred.
May 5, 1917	+	+	P	S	−	−	+	+	−	−	−	
May 17, 1917	+	P	S	−	−	−	+	S	−	−	−	
June 1, 1917	+	P	S	−	−	−	P	S	−	−	−	
June 16, 1917	P	P	S	−	−	−	P	−	−	−	−	
June 29, 1917	P	S	−	−	−	−	P	−	−	−	−	
July 13, 1917	S	S	−	−	−	−	−	−	−	−	−	
July 27, 1917	P	S	−	−	−	−	−	−	−	−	−	
Aug. 11, 1917	S	S	−	−	−	−	−	−	−	−	−	
Aug. 26, 1917	P	−	−	−	−	−	−	−	−	−	−	
Sept. 7, 1917	S	−	−	−	−	−	−	−	−	−	−	
Sept. 21, 1917	−	−	−	−	−	−	−	−	−	−	−	
Oct. 4, 1917	−	−	−	−	−	−	−	−	−	−	−	
Oct. 18, 1917	S	−	−	−	−	−	−	−	−	−	−	
Nov. 1, 1917	S	−	−	−	−	−	−	−	−	−	−	Nov. 2, 1917, received 5 cc. fetal-stomach contents (case 42).
Nov. 14, 1917	S	−	−	−	−	−	−	−	−	−	−	
Nov. 28, 1917	P	−	−	−	−	−	−	−	−	−	−	
Dec. 13, 1917	S	−	−	−	−	−	−	−	−	−	−	
Jan. 4, 1918	P	−	−	−	−	−	−	−	−	−	−	

[a] Explanation of symbols: The plus sign indicates complete clumping of bacteria; P, partial clumping; S, slight trace of agglutination; minus sign, no evidence of clumping.

NOTE.—Figures at head of columns indicate cubic centimeters.

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 402—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Jan. 18, 1918	P	S	–	–	–	–	–	–	–	–	–	Jan. 28, 1918, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta. All 4 pigs when destroyed, Mar. 9, 1919, gave negative results to agglutination test and negative cultural results for *Bact. abortus*.
Feb. 2, 1918	P	–	–	–	–	–	–	–	–	–	–	
Feb. 16, 1918	–	–	–	–	–	–	–	–	–	–	–	
Mar. 1, 1918	S	S	–	–	–	–	–	–	–	–	–	
Mar. 16, 1918	S	S	–	–	–	–	–	–	–	–	–	
Mar. 30, 1918	–	–	–	–	–	–	–	–	–	–	–	
Apr. 13, 1918	S	–	–	–	–	–	–	–	–	–	–	
Apr. 29, 1918	P	–	–	–	–	–	–	–	–	–	–	
May 10, 1918	–	–	–	–	–	–	–	–	–	–	–	
May 25, 1918	–	–	–	–	–	–	–	–	–	–	–	
June 8, 1918	P	–	–	–	–	–	–	–	–	–	–	
June 21, 1918	–	–	–	–	–	–	–	–	–	–	–	
July 6, 1918	–	–	–	–	–	–	–	–	–	–	–	
July 19, 1918	–	–	–	–	–	–	–	–	–	–	–	
Aug. 1, 1918	P	S	–	–	–	–	–	–	–	–	–	
Aug. 16, 1918	P	S	–	–	–	–	–	–	–	–	–	
Aug. 30, 1918	P	S	–	–	–	–	–	–	–	–	–	Aug. 29, 1918, bred.
Sept. 14, 1918	S	–	–	–	–	–	–	–	–	–	–	
Sept. 28, 1918	P	S	–	–	–	–	–	–	–	–	–	
Oct. 12, 1918	S	–	–	–	–	–	–	–	–	–	–	
Oct. 26, 1918	S	–	–	–	–	–	–	–	–	–	–	
Nov. 8, 1918	–	–	–	–	–	–	–	–	–	–	–	Nov. 13, 1918, received 50 cc. fetal-stomach contents (case 74).
Nov. 22, 1918	–	–	–	–	–	–	–	–	–	–	–	
Dec. 6, 1918	–	–	–	–	–	–	–	–	–	–	–	
Dec. 20, 1918	P	–	–	–	–	–	–	–	–	–	–	
Jan. 8, 1919	–	–	–	–	–	–	–	–	–	–	–	
Jan. 22, 1919	S	–	–	–	–	–	–	–	–	–	–	
Feb 7, 1919	P	S	–	–	–	–	–	–	–	–	–	
Feb. 21, 1919	–	–	–	–	–	–	–	–	–	–	–	
Mar. 7, 1919	–	–	–	–	–	–	–	–	–	–	–	
Mar. 21, 1919	P	S	–	–	–	–	–	–	–	–	–	
Apr. 4, 1919	P	P	–	–	–	–	–	–	–	–	–	
Apr. 19, 1919	P	S	–	–	–	–	–	–	–	–	–	
May 3, 1919	P	S	–	–	–	–	–	–	–	–	–	
May 17, 1919	P	S	–	–	–	–	–	–	–	–	–	
May 29, 1919	P	P	–	–	–	–	–	–	–	–	–	June 4, 1919, expelled a living calf. Three guinea pigs inoculated with emulsion of placenta. All 3 pigs, when destroyed, July 9, 1919, gave negative results to agglutination test and negative cultural results for *Bact. abortus*.
June 14, 1919	P	S	–	–	–	–	–	–	–	–	–	
June 25, 1919	P	P	–	–	–	–	–	–	–	–	–	
July 10, 1919	P	–	–	–	–	–	–	–	–	–	–	
July 25, 1919	S	S	–	–	–	–	–	–	–	–	–	
Aug. 9, 1919	P	–	–	–	–	–	–	–	–	–	–	
Aug. 23, 1919	S	–	–	–	–	–	–	–	–	–	–	July 7, 1919, a composite sample of milk gave a negative agglutination reaction. Four inoculated guinea pigs gave negative cultural results for *Bact. abortus* and the 3 which were tested gave negative agglutination results.
Sept. 6, 1919	P	S	–	–	–	–	–	–	–	–	–	
Sept. 19, 1919	P	–	–	–	–	–	–	–	–	–	–	
Oct. 4, 1919	S	–	–	–	–	–	–	–	–	–	–	
Oct. 17, 1919	–	–	–	–	–	–	–	–	–	–	–	
Oct. 31, 1919	P	P	–	–	–	–	–	–	–	–	–	Oct. 3, 1919, bred.
Nov. 14, 1919	P	S	–	–	–	–	–	–	–	–	–	
Nov. 29, 1919	–	–	–	–	–	–	–	–	–	–	–	
Dec. 13, 1919	P	–	–	–	–	–	–	–	–	–	–	
Jan. 3, 1920	–	–	–	–	–	–	–	–	–	–	–	
Jan. 27, 1920	–	–	–	–	–	–	–	–	–	–	–	
Feb. 13, 1920	–	–	–	–	–	–	–	–	–	–	–	Mar. 18, 1920, received 20 cc. fetal-stomach contents (case 110).

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 402—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Feb. 28, 1920	–	–	–	–	–	–	–	–	–	–	–	
Mar. 13, 1920	–	–	–	–	–	–	–	–	–	–	–	
Mar. 27, 1920	–	–	–	–	–	–	–	–	–	–	–	
Apr. 10, 1920	P	S	–	–	–	–	–	–	–	–	–	
Apr. 24, 1920	P	P	–	–	–	–	–	–	–	–	–	
May 7, 1920	P	S	–	–	–	–	–	–	–	–	–	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
May 21, 1920	P	–	–	–	–	–	–	–	–	–	–	
June 5, 1920	S	–	–	–	–	–	–	–	–	–	–	
June 18, 1920	P	–	–	–	–	–	–	–	–	–	–	
July 2, 1920	–	–	–	–	–	–	–	–	–	–	–	July 2, 1920, three guinea pigs injected with milk; when destroyed, Sept. 3, 1920, gave negative cultural results for *Bact. abortus* and negative agglutination reactions.
July 17, 1920	P	–	–	–	–	–	–	–	–	–	–	
July 30, 1920	S	–	–	–	–	–	–	–	–	–	–	
Aug. 13, 1920	S	–	–	–	–	–	–	–	–	–	–	
Aug. 27, 1920	P	–	–	–	–	–	–	–	–	–	–	July 10, 1920, expelled a living calf. Four guinea pigs inoculated with uterine material, when destroyed, Sept. 13, 1920, gave negative cultural results for *Bact. abortus* and negative agglutination reactions.
Sept. 10, 1920	P	–	–	–	–	–	–	–	–	–	–	Sept. 10, 1920, slaughtered. Uterus cultured, negative for *Bact. abortus*. Three guinea pigs, inoculated with milk, when destroyed, Nov. 16, 1920, gave negative cultural results for *Bact. abortus* and negative agglutination results.

VACCINATED HEIFER NO. 409

Feb. 28, 1917	–	–	–	–	–	–	–	–	–	–	–	Mar. 2, 1917, subcutaneous injection of 20 cc. abortion vaccine.
Mar. 7, 1917	+	+	S	–	–	–	–	–	–	–	–	
Mar. 21, 1917	+	+	+	+	+	S	+	+	P	–	–	Mar. 23, 1917, subcutaneous injection 20 cc. abortion vaccine.
Apr. 5, 1917	+	+	+	+	+	S	+	+	+	+	–	
Apr. 21, 1917	+	+	+	+	+	S	+	+	+	+	–	Apr. 3, 1917, bred.
May 5, 1917	+	+	+	P	S	S	+	+	+	P	–	
May 17, 1917	P	+	+	S	–	–	+	+	+	–	–	
June 1, 1917	P	+	P	–	–	–	+	+	S	–	–	June 8, 1917, received 50 cc. fetal-stomach contents (case 21).
June 16, 1917	+	P	S	–	–	–	+	P	–	–	–	
June 29, 1917	+	+	–	–	–	–	+	–	–	–	–	
July 13, 1917	P	S	–	–	–	–	–	–	–	–	–	
July 27, 1917	P	–	–	–	–	–	–	–	–	–	–	
Aug. 11, 1917	+	P	–	–	–	–	–	–	–	–	–	
Aug. 26, 1917	P	–	–	–	–	–	–	–	–	–	–	
Sept. 7, 1917	–	–	–	–	–	–	–	–	–	–	–	
Sept. 21, 1917	–	–	–	–	–	–	–	–	–	–	–	
Oct. 4, 1917	–	–	–	–	–	–	–	–	–	–	–	
Oct. 18, 1917	–	–	–	–	–	–	–	–	–	–	–	
Nov. 1, 1917	–	–	–	–	–	–	–	–	–	–	–	
Nov. 14, 1917	–	–	–	–	–	–	–	–	–	–	–	
Nov. 28, 1917	–	–	–	–	–	–	–	–	–	–	–	
Dec. 13, 1917	–	–	–	–	–	–	–	–	–	–	–	
Jan. 4, 1918	–	–	–	–	–	–	–	–	–	–	–	
Jan. 18, 1918	S	–	–	–	–	–	–	–	–	–	–	Jan. 10, 1918, expelled a living calf. Cultures from placenta negative for *Bact. abortus*. Four guinea pigs inoculation with emulsion of placenta. Two died shortly following inoculation and were not cultured. Two pigs which died Mar. 1 and 9, 1918, respectively, were cultured with negative results for *Bact. abortus*.

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 409—Continued

Date	Serological results: Agglutination						Complement fixation					Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Feb. 2, 1918	–	–	–	–	–	–	–	–	–	–	–	
Feb. 16, 191	S	–	–	–	–	–	–	–	–	–	–	
Mar. 1, 1918	S	–	–	–	–	–	–	–	–	–	–	
Mar. 16, 1918	S	–	–	–	–	–	–	–	–	–	–	
Mar. 30, 1918	–	–	–	–	–	–	–	–	–	–	–	
Apr. 13, 1918	S	–	–	–	–	–	–	–	–	–	–	
Apr. 29, 1918	–	–	–	–	–	–	–	–	–	–	–	
May 10, 1918	–	–	–	–	–	–	–	–	–	–	–	
May 25, 1918	–	–	–	–	–	–	–	–	–	–	–	
June 8, 1918	–	–	–	–	–	–	–	–	–	–	–	
June 21, 1918	–	–	–	–	–	–	–	–	–	–	–	
July 6, 1918	–	–	–	–	–	–	–	–	–	–	–	
July 19, 1918	–	–	–	–	–	–	–	–	–	–	–	July 24, 1918, bred.
Aug. 1, 1918	–	–	–	–	–	–	–	–	–	–	–	
Aug. 16, 1918	–	–	–	–	–	–	–	–	–	–	–	Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
Aug. 30, 1918	–	–	–	–	–	–	–	–	–	–	–	
Sept 14, 1918	–	–	–	–	–	–	–	–	–	–	–	
Sept. 28, 1918	S	–	–	–	–	–	–	–	–	–	–	
Oct. 12, 1918	–	–	–	–	–	–	–	–	–	–	–	
Oct. 25, 1918	S	–	–	–	–	–	–	–	–	–	–	
Nov. 8, 1918	–	–	–	–	–	–	–	–	–	–	–	
Nov. 22, 1918	–	–	–	–	–	–	–	–	–	–	–	
Dec. 6, 1918	–	–	–	–	–	–	–	–	–	–	–	
Dec. 20, 1918	–	–	–	–	–	–	–	–	–	–	–	
Jan. 8, 1919	S	–	–	–	–	–	–	–	–	–	–	
Jan. 22, 1919	–	–	–	–	–	–	–	–	–	–	–	
Feb. 7, 1919	–	–	–	–	–	–	–	–	–	–	–	
Feb. 21, 1919	–	–	–	–	–	–	–	–	–	–	–	
Mar. 7, 1919	–	–	–	–	–	–	–	–	–	–	–	
Mar. 21, 1919	P	–	–	–	–	–	–	–	–	–	–	
Apr. 4, 1919	–	–	–	–	–	–	–	–	–	–	–	
Apr. 19, 1919	P	–	–	–	–	–	–	–	–	–	–	Apr. 30, 1919, expelled a living calf. Five guinea pigs inoculated with an emulsion of placenta. All five pigs when destroyed June 15, 1919, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
May 3, 1919	S	–	–	–	–	–	–	–	–	–	–	
May 17, 1919	–	–	–	–	–	–	–	–	–	–	–	
May 29, 1919	–	–	–	–	–	–	–	–	–	–	–	
June 14, 1919	S	–	–	–	–	–	–	–	–	–	–	
June 25, 1919	–	–	–	–	–	–	–	–	–	–	–	
June 27, 1919	–	–	–	–	–	–	–	–	–	–	–	
July 10, 1919	–	–	–	–	–	–	–	–	–	–	–	
July 25, 1919	–	–	–	–	–	–	–	–	–	–	–	
Aug. 9, 1919	–	–	–	–	–	–	–	–	–	–	–	
Aug. 23, 1919	–	–	–	–	–	–	–	–	–	–	–	
Sept. 6, 1919	–	–	–	–	–	–	–	–	–	–	–	
Sept. 19, 1919	P	–	–	–	–	–	–	–	–	–	–	
Oct. 4, 1919	–	–	–	–	–	–	–	–	–	–	–	
Oct. 17, 1919	–	–	–	–	–	–	–	–	–	–	–	Oct. 24, 1919, bred.
Oct. 31, 1919	–	–	–	–	–	–	–	–	–	–	–	
Nov. 14, 1919	–	–	–	–	–	–	–	–	–	–	–	
Nov. 29, 1919	P	–	–	–	–	–	–	–	–	–	–	
Dec. 13, 1919	S	–	–	–	–	–	–	–	–	–	–	
Jan. 3, 1920	S	S	–	–	–	–	–	–	–	–	–	
Jan. 27, 1920	P	–	–	–	–	–	–	–	–	–	–	
Feb. 13, 1920	S	–	–	–	–	–	–	–	–	–	–	
Feb. 28, 1920	P	–	–	–	–	–	–	–	–	–	–	
Mar 13, 1920	–	–	–	–	–	–	–	–	–	–	–	Mar. 18, 1920, received 20 cc. fetal-stomach contents (case 110).
Mar. 27, 1920	–	–	–	–	–	–	–	–	–	–	–	
Apr. 10, 1920	–	–	–	–	–	–	–	–	–	–	–	
Apr. 24, 1920	P	–	–	–	–	–	–	–	–	–	–	
May 7, 1920	P	–	–	–	–	–	–	–	–	–	–	May 10, 1920, received 20 cc. fetal-stomach contents (case 126).
May 21, 1920	P	–	–	–	–	–	–	–	–	–	–	
June 5, 1920	P	S	–	–	–	–	–	–	–	–	–	
June 18, 1920	S	–	–	–	–	–	–	–	–	–	–	
July 2, 1920	P	–	–	–	–	–	–	–	–	–	–	
July 17, 1920	S	–	–	–	–	–	–	–	–	–	–	

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 409—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
July 30, 1920	P	–	–	–	–	–	–	–	–	–	–	Aug. 4, 1920, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta, when destroyed Sept. 27, 1920, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Aug. 13, 1920	P	–	–	–	–	–	–	–	–	–	–	
Aug. 27, 1920	S	–	–	–	–	–	–	–	–	–	–	
Sept. 10, 1920	S	–	–	–	–	–	–	–	–	–	–	Oct. 10, 1920, destroyed. The uterus, which was cultured, gave negative results for *Bact. abortus*.

VACCINATED HEIFER NO. 417

Feb. 2, 1917	–	–	–	–	–	–	–	–	–	–	–	Feb. 1, 1917, subcutaneous injection 20 cc. abortion vaccine.
Feb. 8, 1917	+	+	+	+	+	–	+	+	+	–	–	
Feb. 19, 1917	+	+	+	+	+	–	+	+	+	–	–	
Mar. 7, 1917	+	+	+	+	–	–	+	+	+	–	–	
Mar. 21, 1917	+	P	–	–	–	–	+	–	–	–	–	
Apr. 5, 1917	+	P	–	–	–	–	+	–	–	–	–	
May 5, 1917	+	P	–	–	–	–	–	–	–	–	–	
May 17, 1917	+	–	–	–	–	–	–	–	–	–	–	
June 1, 1917	+	–	–	–	–	–	–	–	–	–	–	May 25, 1917, bred.
June 16, 1917	P	–	–	–	–	–	–	–	–	–	–	
June 29, 1917	S	–	–	–	–	–	–	–	–	–	–	
July 13, 1917	S	–	–	–	–	–	–	–	–	–	–	
July 27, 1917	P	–	–	–	–	–	–	–	–	–	–	
Aug. 11, 1917	P	–	–	–	–	–	–	–	–	–	–	
Aug. 26, 1917	P	–	–	–	–	–	–	–	–	–	–	
Sept. 7, 1917	S	–	–	–	–	–	–	–	–	–	–	
Sept. 21, 1917	–	–	–	–	–	–	–	–	–	–	–	
Oct. 4, 1917	–	–	–	–	–	–	–	–	–	–	–	
Oct. 18, 1917	S	–	–	–	–	–	–	–	–	–	–	
Nov. 1, 1917	S	–	–	–	–	–	–	–	–	–	–	
Nov. 14, 1917	–	–	–	–	–	–	–	–	–	–	–	
Nov. 28, 1917	S	–	–	–	–	–	–	–	–	–	–	
Dec. 13, 1917	–	–	–	–	–	–	–	–	–	–	–	Dec. 13, 1917, received 30 cc. fetal-stomach contents (case 54).
Jan. 4, 1918	–	–	–	–	–	–	–	–	–	–	–	
Jan. 18, 1918	S	–	–	–	–	–	–	–	–	–	–	
Feb. 2, 1918	S	–	–	–	–	–	–	–	–	–	–	
Feb. 16, 1918	S	–	–	–	–	–	–	–	–	–	–	Feb. 21, 1918, expelled a living calf. Three guinea pigs inoculated with emulsion of placenta, when destroyed May 8, 1918, gave negative agglutination reactions and negative cultural results for *Bact abortus*.
Mar. 1, 1918	P	–	–	–	–	–	–	–	–	–	–	
Mar. 16, 1918	S	–	–	–	–	–	–	–	–	–	–	
Mar. 30, 1918	S	–	–	–	–	–	–	–	–	–	–	
Apr. 13, 1918	S	–	–	–	–	–	–	–	–	–	–	
Apr. 29, 1918	P	S	–	–	–	–	–	–	–	–	–	
May 10, 1918	–	–	–	–	–	–	–	–	–	–	–	
May 25, 1918	S	–	–	–	–	–	–	–	–	–	–	
June 8, 1918	–	–	–	–	–	–	–	–	–	–	–	
June 21, 1918	S	–	–	–	–	–	–	–	–	–	–	
July 6, 1918	–	–	–	–	–	–	–	–	–	–	–	July 12, 1918, bred.
July 19, 1918	–	–	–	–	–	–	–	–	–	–	–	
Aug. 1, 1918	–	–	–	–	–	–	–	–	–	–	–	Aug. 20, 1918, received 30 cc. fetal-stomach contents (case 70).
Aug. 16, 1918	–	–	–	–	–	–	–	–	–	–	–	
Aug. 30, 1918	–	–	–	–	–	–	–	–	–	–	–	
Sept. 14, 1918	–	–	–	–	–	–	–	–	–	–	–	
Sept. 28, 1918	P	–	–	–	–	–	–	–	–	–	–	
Oct. 12, 1918	–	–	–	–	–	–	–	–	–	–	–	
Oct. 25, 1918	–	–	–	–	–	–	–	–	–	–	–	
Nov. 8, 1918	–	–	–	–	–	–	–	–	–	–	–	
Nov. 22, 1918	–	–	–	–	–	–	–	–	–	–	–	
Dec. 6, 1918	–	–	–	–	–	–	–	–	–	–	–	

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 417—Continued

Date	Serological results												Remarks
	Agglutination						Complement fixation						
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002		
Dec. 20, 1918	–	–	–	–	–	–	–	–	–	–	–		
Jan. 8, 1919	S	–	–	–	–	–	–	–	–	–	–		
Jan. 22, 1919	S	–	–	–	–	–	–	–	–	–	–		
Feb. 21, 1919	–	–	–	–	–	–	–	–	–	–	–		
Mar. 7, 1919	–	–	–	–	–	–	–	–	–	–	–		
Mar. 21, 1919	P	–	–	–	–	–	–	–	–	–	–		
Apr. 4, 1919	S	–	–	–	–	–	–	–	–	–	–		Apr. 5, 1919, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta. Two pigs died 3 days later. Two pigs when destroyed June 13, 1919, gave negative agglutination reactions and negative cultural results.
Apr. 19, 1919	P	–	–	–	–	–	–	–	–	–	–		
May 3, 1919	S	–	–	–	–	–	–	–	–	–	–		
May 7, 1919	S	S	–	–	–	–	–	–	–	–	–		
May 29, 1919	P	–	–	–	–	–	–	–	–	–	–		
June 14, 1919	P	–	–	–	–	–	–	–	–	–	–		
June 23, 1919	P	S	–	–	–	–	–	–	–	–	–		
June 27, 1919	P	S	–	–	–	–	–	–	–	–	–		
July 10, 1919	P	–	–	–	–	–	–	–	–	–	–		July 7, 1919, four guinea pigs inoculated with a composite sample of milk. When destroyed Sept. 8, 1919, negative agglutination reactions and negative cultural results for *Bact. abortus* were obtained.
July 25, 1919	S	–	–	–	–	–	–	–	–	–	–		
Aug. 9, 1919	P	S	–	–	–	–	–	–	–	–	–		
Aug. 23, 1919	S	–	–	–	–	–	–	–	–	–	–		
Sept. 6, 1919	–	–	–	–	–	–	–	–	–	–	–		
Sept. 19, 1919	–	–	–	–	–	–	–	–	–	–	–		
Oct. 4, 1919	–	–	–	–	–	–	–	–	–	–	–		
Oct. 17, 1919	S	–	–	–	–	–	–	–	–	–	–		Oct. 24, 1919, bred.
Oct. 31, 1919	S	–	–	–	–	–	–	–	–	–	–		
Nov. 14, 1919	P	–	–	–	–	–	–	–	–	–	–		
Nov. 29, 1919	P	–	–	–	–	–	–	–	–	–	–		
Dec. 13, 1919	P	–	–	–	–	–	–	–	–	–	–		
Jan. 3, 1920	P	–	–	–	–	–	–	–	–	–	–		
Jan. 27, 1920	S	–	–	–	–	–	–	–	–	–	–		
Feb. 13, 1920	–	–	–	–	–	–	–	–	–	–	–		
Feb. 28, 1920	–	–	–	–	–	–	–	–	–	–	–		
Mar. 13, 1920	–	–	–	–	–	–	–	–	–	–	–		Mar. 18, 1920, received 20 cc. fetal-stomach contents (case 110).
Mar. 27, 1920	–	–	–	–	–	–	–	–	–	–	–		
Apr. 10, 1920	P	S	–	–	–	–	–	–	–	–	–		
Apr. 24, 1920	S	S	–	–	–	–	–	–	–	–	–		
May 7, 1920	P	–	–	–	–	–	–	–	–	–	–		May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
May 21, 1920	P	–	–	–	–	–	–	–	–	–	–		
June 5, 1920	S	S	–	–	–	–	–	–	–	–	–		
June 18, 1920	P	–	–	–	–	–	–	–	–	–	–		
July 2, 1920	S	–	–	–	–	–	–	–	–	–	–		July 2, 1920, three guinea pigs inoculated with a composite sample of milk, gave negative agglutination reactions and negative cultural results for *Bact. abortus* when destroyed Sept. 3, 1920.
July 17, 1920	S	–	–	–	–	–	–	–	–	–	–		
July 30, 1920	P	–	–	–	–	–	–	–	–	–	–		
Aug. 15, 1920	P	–	–	–	–	–	–	–	–	–	–		
Aug. 27, 1920	S	–	–	–	–	–	–	–	–	–	–		July 30, 1920, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta gave negative agglutination reactions and negative cultural results for *Bact. abortus* when destroyed Sept. 27, 1920.
Sept. 10, 1920	S	–	–	–	–	–	–	–	–	–	–		

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 418

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Feb. 28, 1917	−	−	−	−	−	−	−	−	−	−	−	Mar. 2, 1917, subcutaneous administration 20 cc. abortion vaccine.
Mar. 7, 1917	+	+	S	−	−	−	−	−	−	−	−	
Mar. 21, 1917	+	+	+	+	+	S	+	+	P	−	−	Mar. 28, 1917, subcutaneous administration 20 cc. abortion vaccine.
Apr. 5, 1917	+	+	+	+	+	S	+	+	+	+	−	May 3, 1917, bred.
Apr. 21, 1917	+	+	+	+	+	S	+	+	+	+	−	
May 5, 1917	+	+	+	F	P	S	+	+	+	P	−	
May 17, 1917	P	+	+	S	−	−	+	+	+	−	−	
June 1, 1917	P	+	P	−	−	−	+	+	S	−	−	
June 16, 1917	+	P	S	−	−	−	+	P	−	−	−	
June 29, 1917	+	+	−	−	−	−	+	−	−	−	−	
July 13, 1917	P	S	−	−	−	−	−	−	−	−	−	
July 27, 1917	P	−	−	−	−	−	−	−	−	−	−	
Aug. 11, 1917	+	P	−	−	−	−	−	−	−	−	−	
Aug. 26, 1917	P	−	−	−	−	−	−	−	−	−	−	
Sept. 7, 1917	−	−	−	−	−	−	−	−	−	−	−	
Sept. 21, 1917	−	−	−	−	−	−	−	−	−	−	−	
Oct. 4, 1917	−	−	−	−	−	−	−	−	−	−	−	
Oct. 18, 1917	−	−	−	−	−	−	−	−	−	−	−	
Nov. 1, 1917	−	−	−	−	−	−	−	−	−	−	−	
Nov. 14, 1917	−	−	−	−	−	−	−	−	−	−	−	
Nov. 28, 1917	−	−	−	−	−	−	−	−	−	−	−	
Dec. 13, 1917	−	−	−	−	−	−	−	−	−	−	−	
Jan. 4, 1918	−	−	−	−	−	−	−	−	−	−	−	
Jan. 18, 1918	S	−	−	−	−	−	−	−	−	−	−	
Feb. 2, 1918	−	−	−	−	−	−	−	−	−	−	−	Feb. 5, 1918, expelled a living calf. Three guinea pigs inoculated with an emulsion of placenta. One pig died four days later. Two pigs which were slaughtered Mar. 13, 1918, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Feb. 16, 1918	S	−	−	−	−	−	−	−	−	−	−	
Mar. 1, 1918	S	−	−	−	−	−	−	−	−	−	−	
Mar. 16, 1918	S	−	−	−	−	−	−	−	−	−	−	
Mar. 30, 1918	−	−	−	−	−	−	−	−	−	−	−	
Apr. 13, 1918	S	−	−	−	−	−	−	−	−	−	−	
Apr. 29, 1918	−	−	−	−	−	−	−	−	−	−	−	
May 10, 1918	−	−	−	−	−	−	−	−	−	−	−	
May 25, 1918	−	−	−	−	−	−	−	−	−	−	−	
June 8, 1918	−	−	−	−	−	−	−	−	−	−	−	
June 21, 1918	−	−	−	−	−	−	−	−	−	−	−	
July 6, 1918	−	−	−	−	−	−	−	−	−	−	−	
July 19, 1918	−	−	−	−	−	−	−	−	−	−	−	July 25, 1918, bred.
Aug. 1, 1918	−	−	−	−	−	−	−	−	−	−	−	
Aug. 16, 1918	S	−	−	−	−	−	−	−	−	−	−	Aug. 20, 1918, received 25 cc. fetal stomach contents (case 70).
Aug. 30, 1918	−	−	−	−	−	−	−	−	−	−	−	
Sept. 14, 1918	−	−	−	−	−	−	−	−	−	−	−	
Sept. 28, 1918	S	−	−	−	−	−	−	−	−	−	−	
Oct. 12, 1918	−	−	−	−	−	−	−	−	−	−	−	
Oct. 25, 1918	S	−	−	−	−	−	−	−	−	−	−	
Nov. 8, 1918	−	−	−	−	−	−	−	−	−	−	−	
Nov. 22, 1918	−	−	−	−	−	−	−	−	−	−	−	
Dec. 6, 1918	−	−	−	−	−	−	−	−	−	−	−	
Dec. 20, 1918	−	−	−	−	−	−	−	−	−	−	−	
Jan. 6, 1919	S	−	−	−	−	−	−	−	−	−	−	
Jan. 22, 1919	−	−	−	−	−	−	−	−	−	−	−	
Feb. 7, 1919	−	−	−	−	−	−	−	−	−	−	−	
Feb. 21, 1919	−	−	−	−	−	−	−	−	−	−	−	
Mar. 7, 1919	−	−	−	−	−	−	−	−	−	−	−	
Mar. 21, 1919	P	−	−	−	−	−	−	−	−	−	−	
Apr. 4, 1919	−	−	−	−	−	−	−	−	−	−	−	
Apr. 19, 1919	P	−	−	−	−	−	−	−	−	−	−	
May 3, 1919	S	−	−	−	−	−	−	−	−	−	−	May 5, 1919, expelled a living calf. Four guinea pigs inoculated with uterine material. When destroyed June 13, 1919, all gave negative agglutination reactions and negative cultural results for *Bact. abortus*.

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 418—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
May 17, 1919	−	−	−	−	−	−	−	−	−	−	−	
May 29, 1919	−	−	−	−	−	−	−	−	−	−	−	
June 14, 1919	S	−	−	−	−	−	−	−	−	−	−	
June 25, 1919	−	−	−	−	−	−	−	−	−	−	−	
July 10, 1919	−	−	−	−	−	−	−	−	−	−	−	May 5, 1919, a composite sample of milk obtained and four guinea pigs inoculated. Three pigs when destroyed June 13, 1919, gave negative agglutination reactions and negative cultural results for *Bact. abortus*. Other pig died soon after inoculation.
July 25, 1919	−	−	−	−	−	−	−	−	−	−	−	
Aug. 9, 1919	−	−	−	−	−	−	−	−	−	−	−	
Aug. 23, 1919	−	−	−	−	−	−	−	−	−	−	−	
Sept. 6, 1919	−	−	−	−	−	−	−	−	−	−	−	
Sept. 19, 1919	−	−	−	−	−	−	−	−	−	−	−	
Oct. 4, 1919	−	−	−	−	−	−	−	−	−	−	−	July 7, 1919, four guinea pigs inoculated with a composite sample of milk; when destroyed Aug. 22, 1920, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Oct. 17, 1919	−	−	−	−	−	−	−	−	−	−	−	
Oct. 31, 1919	S	−	−	−	−	−	−	−	−	−	−	
Nov. 14, 1919	−	−	−	−	−	−	−	−	−	−	−	
Nov. 29, 1919	P	−	−	−	−	−	−	−	−	−	−	Oct. 8, 1919, bred.
Dec. 13, 1919	−	−	−	−	−	−	−	−	−	−	−	
Jan. 3, 1920	−	−	−	−	−	−	−	−	−	−	−	
Jan. 27, 1920	−	−	−	−	−	−	−	−	−	−	−	
Feb. 13, 1920	−	−	−	−	−	−	−	−	−	−	−	
Feb. 28, 1920	−	−	−	−	−	−	−	−	−	−	−	
Mar. 13, 1920	−	−	−	−	−	−	−	−	−	−	−	Mar 18, 1920, received 20 cc. fetal-stomach contents (case 110).
Mar. 27, 1920	−	−	−	−	−	−	−	−	−	−	−	
Apr. 10, 1920	−	−	−	−	−	−	−	−	−	−	−	
Apr. 24, 1920	−	−	−	−	−	−	−	−	−	−	−	
May 7, 1920	−	−	−	−	−	−	−	−	−	−	−	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
May 21, 1920	−	−	−	−	−	−	−	−	−	−	−	
June 5, 1920	−	−	−	−	−	−	−	−	−	−	−	
June 18, 1920	−	−	−	−	−	−	−	−	−	−	−	
July 2, 1920	−	−	−	−	−	−	−	−	−	−	−	July 2, 1920, three guinea pigs inoculated with a composite sample of milk; gave negative agglutination reactions and negative cultural results for *Bact. abortus* when destroyed Sept. 13, 1920.
July 17, 1920	S	−	−	−	−	−	−	−	−	−	−	
July 30, 1920	−	−	−	−	−	−	−	−	−	−	−	
Aug. 13, 1920	−	−	−	−	−	−	−	−	−	−	−	
Aug. 27, 1920	−	−	−	−	−	−	−	−	−	−	−	July 12, 1920, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta; when destroyed Sept. 13, 1920, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Sept. 10, 1920	−	−	−	−	−	−	−	−	−	−	−	

VACCINATED HEIFER NO. 419

Feb. 28, 1918	−	−	−	−	−	−	−	−	−	−	−	Mar. 30, 1918, subcutaneous administration 20 cc. abortion vaccine.
Apr. 13, 1918	+	+	+	+	P	S	+	+	+	−	−	
Apr. 29, 1918	+	+	+	+	P	−	+	+	P	−	−	
May 10, 1918	+	P	P	P	−	−	+	+	+	−	−	
May 25, 1918	+	P	P	−	−	−	+	S	−	−	−	
June 8, 1918	+	P	S	−	−	−	+	−	−	−	−	
June 21, 1918	P	P	−	−	−	−	−	−	−	−	−	
July 6, 1918	P	P	−	−	−	−	−	−	−	−	−	
July 19, 1918	P	S	−	−	−	−	−	−	−	−	−	
Aug. 1, 1918	P	−	−	−	−	−	−	−	−	−	−	

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 419—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Aug. 16, 1918	P	P	–	–	–	–	–	–	–	–	–	
Aug. 30, 1918	P	P	–	–	–	–	–	–	–	–	–	
Sept. 14, 1918	P	S	–	–	–	–	–	–	–	–	–	
Sept. 28, 1918	P	P	–	–	–	–	–	–	–	–	–	
Oct. 12, 1918	+	P	–	–	–	–	–	–	–	–	–	
Oct. 26, 1918	P	P	–	–	–	–	–	–	–	–	–	
Nov. 8, 1918	P	–	–	–	–	–	–	–	–	–	–	
Nov. 22, 1918	P	S	–	–	–	–	–	–	–	–	–	
Dec. 6, 1918	P	–	–	–	–	–	–	–	–	–	–	
Dec. 20, 1918	S	S	–	–	–	–	–	–	–	–	–	Dec. 30, 1918, bred.
Jan. 8, 1919	P	–	–	–	–	–	–	–	–	–	–	
Jan. 22, 1919	P	P	–	–	–	–	–	–	–	–	–	
Feb. 7, 1919	P	P	–	–	–	–	–	–	–	–	–	
Feb. 21, 1919	P	S	–	–	–	–	–	–	–	–	–	Feb. 24, 1919, received 60 cc. fetal-stomach contents (case 80).
Mar. 7, 1919	P	P	–	–	–	–	–	–	–	–	–	
Mar. 21, 1919	P	S	–	–	–	–	–	–	–	–	–	Mar. 19, 1919, received 25 cc. fetal-stomach contents (case 88).
Apr. 4, 1919	+	P	–	–	–	–	–	–	–	–	–	
Apr. 19, 1919	P	P	–	–	–	–	–	–	–	–	–	
May 3, 1919	P	P	–	–	–	–	–	–	–	–	–	
May 17, 1919	P	P	S	–	–	–	–	–	–	–	–	
May 29, 1919	P	S	–	–	–	–	–	–	–	–	–	
June 14, 1919	P	P	S	–	–	–	–	–	–	–	–	
June 25, 1919	P	P	S	–	–	–	–	–	–	–	–	
July 10, 1919	+	P	S	–	–	–	–	–	–	–	–	
July 25, 1919	+	S	–	–	–	–	–	–	–	–	–	
Aug. 9, 1919	P	P	–	–	–	–	–	–	–	–	–	
Aug. 23, 1919	P	P	–	–	–	–	–	–	–	–	–	
Sept. 6, 1919	P	S	–	–	–	–	–	–	–	–	–	
Sept. 19, 1919	P	–	–	–	–	–	–	–	–	–	–	
Oct. 4, 1919	P	P	–	–	–	–	–	–	–	–	–	Oct. 7, 1919, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta. When destroyed, Dec. 1, 1919, negative agglutination reactions and negative cultural results for *Bact. abortus* obtained.
Oct. 17, 1919	P	S	–	–	–	–	–	–	–	–	–	
Oct. 31, 1919	+	S	–	–	–	–	–	–	–	–	–	
Nov. 14, 1919	P	S	–	–	–	–	–	–	–	–	–	
Nov. 29, 1919	P	S	–	–	–	–	–	–	–	–	–	
Dec. 13, 1919	P	S	–	–	–	–	–	–	–	–	–	
Jan. 3, 1920	P	–	–	–	–	–	–	–	–	–	–	
Jan. 27, 1920	S	S	–	–	–	–	–	–	–	–	–	
Feb. 13, 1920	S	S	–	–	–	–	–	–	–	–	–	
Feb. 28, 1920	P	S	–	–	–	–	–	–	–	–	–	
Mar. 13, 1920	P	S	–	–	–	–	–	–	–	–	–	
Mar. 27, 1920	S	S	–	–	–	–	–	–	–	–	–	
Apr. 10, 1920	P	S	–	–	–	–	–	–	–	–	–	
Apr. 24, 1920	P	P	–	–	–	–	–	–	–	–	–	Apr. 21, 1920, bred.
May 7, 1920	P	P	–	–	–	–	–	–	–	–	–	
May 21, 1920	S	S	–	–	–	–	–	–	–	–	–	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
June 5, 1920	P	–	–	–	–	–	–	–	–	–	–	
June 18, 1920	P	P	–	–	–	–	–	–	–	–	–	
July 2, 1920	P	P	S	–	–	–	–	–	–	–	–	July 2, 1920, three guinea pigs inoculated with a composite sample of milk; when destroyed, Sept. 3, 1920, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
July 17, 1920	P	P	–	–	–	–	–	–	–	–	–	
July 30, 1920	P	S	S	–	–	–	–	–	–	–	–	
Aug. 13, 1920	P	S	S	–	–	–	–	–	–	–	–	
Aug. 27, 1920	P	P	S	–	–	–	–	–	–	–	–	
Sept. 10, 1920	P	S	S	–	–	–	–	–	–	–	–	
Sept. 25, 1920	+	P	S	–	–	–	–	–	–	–	–	
Oct. 7, 1920	P	P	–	–	–	–	–	–	–	–	–	
Oct. 22, 1920	P	S	–	–	–	–	–	–	–	–	–	
Nov. 8, 1920	P	S	–	–	–	–	–	–	–	–	–	
Nov. 27, 1920	S	S	–	–	–	–	–	–	–	–	–	
Dec. 11, 1920	S	S	–	–	–	–	–	–	–	–	–	
Dec. 29, 1920	P	S	–	–	–	–	–	–	–	–	–	

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 419—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks.
Jan. 20, 1921	S	S	–	–	–	–	–	–	–	–	–	Jan. 27, 1921, expelled a living calf. Three guinea pigs inoculated with emulsion of placenta; when destroyed, Mar. 30, 1920, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Feb. 14, 1921	P	–	–	–	–	–	–	–	–	–	–	
Mar. 3, 1921	P	–	–	–	–	–	–	–	–	–	–	

VACCINATED HEIFER NO. 427

Feb. 26, 1917	–	–	–	–	–	–	–	–	–	–	–	Mar. 2, 1917, subcutaneous administration 20 cc. abortion vaccine.
Mar. 7, 1917	S	–	–	–	–	–	–	–	–	–	–	
Mar. 21, 1917	+	+	+	+	S	–	+	+	–	–	–	Apr. 6, 1917, bred.
Apr. 5, 1917	+	+	+	+	S	–	+	+	S	–	–	
Apr. 21, 1917	+	+	+	+	S	–	+	+	+	–	–	
May 5, 1917	+	+	P	S	–	–	+	+	+	–	–	
May 11, 1917	P	P	S	–	–	–	+	P	–	–	–	
June 1, 1917	P	S	S	–	–	–	+	–	–	–	–	June 8, 1917, received 50 cc. fetal stomach contents (case 21).
June 16, 1917	P	S	–	–	–	–	S	–	–	–	–	
June 29, 1917	P	S	–	–	–	–	–	–	–	–	–	
July 13, 1917	P	S	–	–	–	–	–	–	–	–	–	
July 27, 1917	P	S	–	–	–	–	–	–	–	–	–	
Aug. 11, 1917	P	S	–	–	–	–	–	–	–	–	–	
Aug. 26, 1917	P	S	–	–	–	–	–	–	–	–	–	
Sept. 7, 1917	P	–	–	–	–	–	–	–	–	–	–	
Sept. 21, 1917	S	–	–	–	–	–	–	–	–	–	–	
Oct 4, 1917	–	–	–	–	–	–	–	–	–	–	–	
Oct. 18, 1917	–	–	–	–	–	–	–	–	–	–	–	
Nov. 1, 1917	–	–	–	–	–	–	–	–	–	–	–	
Nov. 14, 1917	–	–	–	–	–	–	–	–	–	–	–	
Nov. 28, 1917	S	–	–	–	–	–	–	–	–	–	–	
Dec. 13, 1917	S	–	–	–	–	–	–	–	–	–	–	
Jan. 4, 1918	–	–	–	–	–	–	–	–	–	–	–	
Jan. 18, 1918	P	–	–	–	–	–	–	–	–	–	–	Jan. 13, 1918, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta. All died after a period of six weeks or longer. Cultural results negative for *Bact. abortus*.
Feb. 2, 1918	P	–	–	–	–	–	–	–	–	–	–	
Feb. 16, 1918	S	–	–	–	–	–	–	–	–	–	–	
Mar. 1, 1918	S	–	–	–	–	–	–	–	–	–	–	
Mar. 16, 1918	–	–	–	–	–	–	–	–	–	–	–	
Mar. 30, 1918	P	–	–	–	–	–	–	–	–	–	–	
Apr. 13, 1918	S	–	–	–	–	–	–	–	–	–	–	
Apr. 29, 1918	P	–	–	–	–	–	–	–	–	–	–	
May 10, 1918	P	–	–	–	–	–	–	–	–	–	–	
May 25, 1918	S	–	–	–	–	–	–	–	–	–	–	
June 8, 1918	P	S	–	–	–	–	–	–	–	–	–	
June 21, 1918	S	–	–	–	–	–	–	–	–	–	–	
July 6, 1918	P	–	–	–	–	–	–	–	–	–	–	
July 19, 1918	S	–	–	–	–	–	–	–	–	–	–	July 17, 1918, bred.
Aug. 1, 1918	P	–	–	–	–	–	–	–	–	–	–	
Aug. 16, 1918	S	–	–	–	–	–	–	–	–	–	–	Aug. 20, 1918, received 25 cc. fetal stomach contents (case 70).
Aug. 30, 1918	–	–	–	–	–	–	–	–	–	–	–	
Sept. 14, 1918	–	–	–	–	–	–	–	–	–	–	–	
Sept. 28, 1918	–	–	–	–	–	–	–	–	–	–	–	
Oct. 12, 1918	S	–	–	–	–	–	–	–	–	–	–	
Oct. 25, 1918	P	–	–	–	–	–	–	–	–	–	–	
Nov. 8, 1918	P	–	–	–	–	–	–	–	–	–	–	
Nov. 22, 1918	–	–	–	–	–	–	–	–	–	–	–	
Dec. 6, 1918	–	–	–	–	–	–	–	–	–	–	–	
Dec. 20, 1918	P	–	–	–	–	–	–	–	–	–	–	
Jan. 8, 1919	–	–	–	–	–	–	–	–	–	–	–	
Jan. 22, 1919	S	S	–	–	–	–	–	–	–	–	–	
Feb. 7, 1919	P	S	–	–	–	–	–	–	–	–	–	
Feb. 21, 1919	–	–	–	–	–	–	–	–	–	–	–	
Mar. 7, 1919	–	–	–	–	–	–	–	–	–	–	–	
Mar. 21, 1919	P	–	–	–	–	–	–	–	–	–	–	

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 427—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.00	Complement fixation 50.00	20.00	10.00	40.00	20.00	10.00	50.00	Remarks
Apr. 4, 1919	–	–	–	–	–	–	–	–	–	–	–	Apr. 21, 1919, expelled a living calf. Three guinea pigs inoculated with uterine material. When destroyed, June 13, 1919, negative agglutination reactions and negative cultural results for *Bact. abortus* were obtained.
Apr. 19, 1919	P	–	–	–	–	–	–	–	–	–	–	
May 3, 1919	P	–	–	–	–	–	–	–	–	–	–	
May 27, 1919	–	–	–	–	–	–	–	–	–	–	–	
May 23, 1919	–	–	–	–	–	–	–	–	–	–	–	
June 14, 1919	P	S	–	–	–	–	–	–	–	–	–	Apr. 21, 1919, three guinea pigs inoculated with a sample of milk. When destroyed, June 13, 1919, negative agglutination reactions and negative cultural results obtained.
June 25, 1919	P	S	–	–	–	–	–	–	–	–	–	
July 10, 1919	–	–	–	–	–	–	–	–	–	–	–	
July 25, 1919	S	–	–	–	–	–	–	–	–	–	–	
Aug. 9, 1919	P	S	–	–	–	–	–	–	–	–	–	July 7, 1919, four guinea pigs inoculated with a composite sample of milk. When destroyed, Sept. 8, 1919, negative agglutination reactions and negative cultural results for *Bact. abortus* obtained.
Aug. 23, 1919	P	S	–	–	–	–	–	–	–	–	–	
Sept. 6, 1919	–	–	–	–	–	–	–	–	–	–	–	
Sept. 19, 1919	–	–	–	–	–	–	–	–	–	–	–	
Oct. 4, 1919	–	–	–	–	–	–	–	–	–	–	–	Oct. 3, 1919, bred.
Oct. 17, 1919	–	–	–	–	–	–	–	–	–	–	–	
Oct. 31, 1919	–	–	–	–	–	–	–	–	–	–	–	
Nov. 14, 1919	–	–	–	–	–	–	–	–	–	–	–	
Nov. 29, 1919	P	S	–	–	–	–	–	–	–	–	–	
Dec. 13, 1919	–	–	–	–	–	–	–	–	–	–	–	
Jan. 3, 1920	–	–	–	–	–	–	–	–	–	–	–	
Jan. 27, 1920	S	–	–	–	–	–	–	–	–	–	–	
Feb. 13, 1920	–	–	–	–	–	–	–	–	–	–	–	
Feb. 26, 1920	–	–	–	–	–	–	–	–	–	–	–	
Mar. 13, 1920	–	–	–	–	–	–	–	–	–	–	–	Mar. 18, 1920, received 20 cc. fetal stomach contents (case 119).
Mar. 27, 1920	–	–	–	–	–	–	–	–	–	–	–	
Apr. 10, 1920	P	S	–	–	–	–	–	–	–	–	–	
Apr. 24, 1920	P	S	–	–	–	–	–	–	–	–	–	
May 7, 1920	S	S	–	–	–	–	–	–	–	–	–	May 10, 1920, received 22 cc. fetal stomach contents (case 126).
May 21, 1920	S	–	–	–	–	–	–	–	–	–	–	
June 5, 1920	–	–	–	–	–	–	–	–	–	–	–	
June 18, 1920	–	–	–	–	–	–	–	–	–	–	–	
July 2, 1920	P	–	–	–	–	–	–	–	–	–	–	July 2, 1920, three guinea pigs inoculated with a composite sample of milk. When destroyed, Sept. 13, 1920, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
July 17, 1920	P	–	–	–	–	–	–	–	–	–	–	
July 30, 1920	S	–	–	–	–	–	–	–	–	–	–	
Aug. 13, 1920	–	–	–	–	–	–	–	–	–	–	–	
Aug. 27, 1920	S	–	–	–	–	–	–	–	–	–	–	July 12, 1920, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta; when destroyed, Sept. 13, 1920, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 462

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Sept. 10, 1920	−	−	−	−	−	−	−	−	−	−	−	
Jan. 8, 1919	−	−	−	−	−	−	−	−	−	−	−	Jan. 10,1919, subcutaneous administration 20 cc. abortion vaccine.
Jan. 22, 1919	+	+	S	−	−	−	+	−	−	−	−	
Feb. 7, 1919	+	+	+	+	P	−	+	+	+	+	−	
Feb. 21, 1919	+	+	+	P	P	−	+	+	P	−	−	
Mar. 7, 1919	+	+	+	P	−	−	+	+	−	−	−	Mar. 12, 1919, bred.
Mar. 21, 1919	+	+	+	P	−	−	+	+	−	−	−	
Apr. 4, 1919	+	+	P	−	−	−	+	P	−	−	−	
Apr. 19, 1919	+	P	S	−	−	−	+	−	−	−	−	
May 3, 1919	+	P	−	−	−	−	−	−	−	−	−	
May 17, 1919	+	P	−	−	−	−	−	−	−	−	−	May 24, 1919, received 50 cc fetal-stomach contents (case 92).
May 29, 1919	+	P	−	−	−	−	−	−	−	−	−	
June 14, 1919	P	P	−	−	−	−	−	−	−	−	−	
June 25, 1919	P	S	−	−	−	−	−	−	−	−	−	
July 10, 1919	P	P	−	−	−	−	−	−	−	−	−	
July 25, 1919	P	S	−	−	−	−	−	−	−	−	−	
Aug. 9, 1919	P	−	−	−	−	−	−	−	−	−	−	
Aug. 23, 1919	P	S	−	−	−	−	−	−	−	−	−	
Sept. 6, 1919	S	−	−	−	−	−	−	−	−	−	−	
Sept. 19, 1919	P	−	−	−	−	−	−	−	−	−	−	
Oct. 4, 1919	P	−	−	−	−	−	−	−	−	−	−	
Oct. 17, 1919	S	−	−	−	−	−	−	−	−	−	−	
Oct. 31, 1919	−	−	−	−	−	−	−	−	−	−	−	
Nov. 14, 1919	−	−	−	−	−	−	−	−	−	−	−	
Nov. 29, 1919	P	S	−	−	−	−	−	−	−	−	−	
Dec. 13, 1919	P	S	−	−	−	−	−	−	−	−	−	Dec. 22, 1919, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta. All died during following month. Cultural results negative for *Bact. abortus*.
Jan. 3, 1920	S	S	−	−	−	−	−	−	−	−	−	
Jan. 27, 1920	S	−	−	−	−	−	−	−	−	−	−	
Feb. 13, 1920	S	−	−	−	−	−	−	−	−	−	−	
Feb. 28, 1920	−	−	−	−	−	−	−	−	−	−	−	
Mar. 13, 1920	−	−	−	−	−	−	−	−	−	−	−	
Mar. 27, 1920	P	−	−	−	−	−	−	−	−	−	−	
Apr. 10, 1920	P	S	−	−	−	−	−	−	−	−	−	
Apr. 21, 1920	P	S	−	−	−	−	−	−	−	−	−	Apr. 26, 1920, bred.
May 7, 1920	P	−	−	−	−	−	−	−	−	−	−	
May 21, 1920	S	−	−	−	−	−	−	−	−	−	−	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
June 5, 1920	P	S	−	−	−	−	−	−	−	−	−	
June 18, 1920	S	S	−	−	−	−	−	−	−	−	−	
July 2, 1920	P	S	−	−	−	−	−	−	−	−	−	July 2, 1920, three guinea pigs inoculated with a composite sample of milk. When destroyed, Sept. 3, 1920, negative agglutination reactions and negative cultural results for *Bact. abortus* obtained.
July 17, 1920	S	−	−	−	−	−	−	−	−	−	−	
July 30, 1920	S	S	−	−	−	−	−	−	−	−	−	
Aug. 13, 1920	S	−	−	−	−	−	−	−	−	−	−	
Aug. 27, 1920	−	−	−	−	−	−	−	−	−	−	−	
Sept. 10, 1920	−	−	−	−	−	−	−	−	−	−	−	
Sept. 25, 1920	−	−	−	−	−	−	−	−	−	−	−	
Oct. 7, 1920	−	−	−	−	−	−	−	−	−	−	−	
Oct. 22, 1920	S	−	−	−	−	−	−	−	−	−	−	
Nov. 8, 1920	−	−	−	−	−	−	−	−	−	−	−	
Nov. 27, 1920	P	−	−	−	−	−	−	−	−	−	−	Feb. 4, 1921, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta; when destroyed, Mar. 22, 1921, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Dec. 11, 1920	−	−	−	−	−	−	−	−	−	−	−	
Dec. 29, 1920	−	−	−	−	−	−	−	−	−	−	−	
Jan. 20, 1921	−	−	−	−	−	−	−	−	−	−	−	
Feb. 14, 1921	−	−	−	−	−	−	−	−	−	−	−	
Mar. 3, 1921	S	−	−	−	−	−	−	−	−	−	−	

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 447

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Feb. 28, 1918	−	−	−	−	−	−	−	−	−	−	−	
Mar. 16, 1918	−	−	−	−	−	−	−	−	−	−	−	Mar. 30, 1918, subcutaneous administration 20 cc. abortion vaccine.
Apr. 13, 1918	+	+	+	+	+	P	+	+	+	+	+	
Apr. 29, 1918	+	+	+	+	P	S	+	+	+	+	−	
May 10, 1918	+	+	+	P	P	−	+	+	+	−	−	
May 25, 1918	+	+	P	P	−	−	+	+	P	−	−	
June 8, 1918	+	P	P	−	−	−	+	P	−	−	−	
June 21, 1918	+	P	S	−	−	−	+	S	−	−	−	July 1, 1918, bred.
July 6, 1918	+	S	S	−	−	−	+	−	−	−	−	
July 19, 1918	P	S	−	−	−	−	−	−	−	−	−	
Aug. 1, 1918	P	S	−	−	−	−	−	−	−	−	−	
Aug. 16, 1918	P	P	−	−	−	−	−	−	−	−	−	
Aug. 30, 1918	P	S	−	−	−	−	−	−	−	−	−	
Sept. 14, 1918	P	P	−	−	−	−	−	−	−	−	−	
Sept. 28, 1918	S	S	−	−	−	−	−	−	−	−	−	
Oct. 12, 1918	P	−	−	−	−	−	−	−	−	−	−	
Oct. 25, 1918	S	−	−	−	−	−	−	−	−	−	−	Nov. 13, 1918, received 30 cc. fetal-stomach contents (case 74).
Nov. 8, 1918	S	−	−	−	−	−	−	−	−	−	−	
Nov. 22, 1918	−	−	−	−	−	−	−	−	−	−	−	
Dec. 6, 1918	S	−	−	−	−	−	−	−	−	−	−	
Dec. 20, 1918	P	−	−	−	−	−	−	−	−	−	−	
Jan. 8, 1919	P	S	−	−	−	−	−	−	−	−	−	
Jan. 22, 1919	S	S	−	−	−	−	−	−	−	−	−	
Feb. 7, 1919	P	−	−	−	−	−	−	−	−	−	−	
Feb. 21, 1919	P	P	S	−	−	−	−	−	−	−	−	
Mar. 7, 1919	+	+	+	P	P	−	+	P	−	−	−	Mar. 10, 1919, three guinea pigs inoculated with vaginal material and three with colostrum; when destroyed, June 13, 1919, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Mar. 21, 1919	+	+	+	+	+	+	+	+	+	+	−	
Apr. 4, 1919	+	+	+	+	+	+	+	+	+	+	P	
Apr. 19, 1919	+	+	+	+	+	+	+	+	+	+	+	Mar. 17, 1919, expelled a dead fetus. *Bact. abortus* isolated from both fetus and placenta by cultural methods.

VACCINATED COW NO. 408

Feb. 7, 1919	−	−	−	−	−	−	−	−	−	−	−	
Feb. 21, 1919	−	−	−	−	−	−	−	−	−	−	−	Feb. 24, 1919, subcutaneous administration 20 cc. abortion vaccine.
Mar. 7, 1919	+	+	+	+	P	−	+	+	+	−	−	
Mar. 21, 1919	+	+	+	+	+	S	+	+	+	S	−	
Apr. 4, 1919	+	+	+	+	P	−	+	+	+	−	−	Apr. 12, 1919, bred.
Apr. 19, 1919	+	+	+	+	S	−	+	+	P	−	−	
May 3, 1919	+	+	+	+	S	−	+	+	S	−	−	
May 17, 1919	+	+	+	P	−	−	+	+	P	−	−	May 24, 1919, received 50 cc. fetal stomach contents (case 92).
May 29, 1919	+	+	+	S	−	−	+	+	S	−	−	
June 14, 1919	+	+	+	S	−	−	+	+	−	−	−	
June 25, 1919	+	+	+	−	−	−	+	P	−	−	−	
July 10, 1919	+	+	P	−	−	−	+	P	−	−	−	
July 25, 1919	+	+	S	−	−	−	P	−	−	−	−	
Aug. 9, 1919	+	P	S	−	−	−	−	−	−	−	−	
Aug. 20, 1919	+	P	−	−	−	−	−	−	−	−	−	
Sept. 6, 1919	P	S	−	−	−	−	−	−	−	−	−	
Sept. 19, 1919	P	S	−	−	−	−	−	−	−	−	−	
Oct. 4, 1919	P	P	−	−	−	−	−	−	−	−	−	
Oct. 17, 1919	+	P	S	−	−	−	−	−	−	−	−	
Oct. 31, 1919	+	+	P	S	−	−	+	+	P	−	−	
Nov. 4, 1919	P	P	+	+	+	+	+	+	+	+	P	
Nov. 14, 1919	P	P	+	+	+	+	+	+	+	+	+	Nov. 20, 1919, three guinea pigs inoculated with a composite sample of milk. When destroyed, Jan. 7, 1920, gave positive agglutination reactions. *Bact. abortus* isolated from all pigs.

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED COW NO. 408—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Nov. 29, 1919	P	P	+	+	+	+	+	+	+	+	+	
Dec. 13, 1919	P	P	+	+	+	+	+	+	+	+	+	
Jan. 3, 1920	P	+	+	+	+	+	+	+	+	+	+	
Jan. 27, 1920	P	P	+	+	+	+	+	+	+	+	+	Jan. 20, 1920, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta. When destroyed, Mar. 16, 1920, negative agglutination reactions and negative cultural results for *Bact. abortus* obtained.
Feb. 13, 1920	+	+	+	+	+	+	+	+	+	+	−	
Feb. 28, 1920	+	+	+	+	P	P	+	+	+	+	−	
Mar. 13, 1920	+	+	+	+	P	S	+	+	+	+	−	
Mar. 27, 1920	+	+	+	+	P	P	+	+	+	+	S	
Apr. 10, 1920	+	+	+	+	+	P	+	+	+	+	−	
Apr. 24, 1920	+	+	+	+	+	S	+	+	+	+	−	
May 7, 1920	+	+	+	+	+	−	+	+	+	P	−	
May 21, 1920	+	+	+	+	+	S	+	+	+	+	−	
June 5, 1920	+	+	+	+	+	P	+	+	+	+	−	June 7, 1920, three guinea pigs inoculated with milk sediment. When destroyed, Aug. 1, 1920, gave positive agglutination reactions and positive cultural results for *Bact. abortus*.
June 18, 1920	+	+	+	+	+	P	+	+	+	+	−	

VACCINATED COW NO. 410

Jan. 29, 1920	−	−	−	−	−	−	−	−	−	−	−	Mar. 2, 1920, subcutaneous administration 20 cc. abortion vaccine.
Feb. 13, 1920	−	−	−	−	−	−	−	−	−	−	−	Mar. 19, 1920, three guinea pigs inoculated with milk. When destroyed, May 17, 1920, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Feb. 28, 1920	−	−	−	−	−	−	−	−	−	−	−	
Mar. 13, 1920	+	P	S	−	−	S	+	−	−	−	−	
Mar. 27, 1920	+	+	+	+	S	S	+	+	+	P	−	Apr. 15, 1920, four guinea pigs inoculated with milk. When destroyed, June 5, 1920, 2 pigs gave positive agglutination reactions. *Bact. abortus* isolated from pigs.
Apr. 10, 1920	+	+	+	+	+	S	+	+	+	P	−	
Apr. 24, 1920	P	P	+	+	+	P	+	+	+	+	−	
May 7, 1920	P	P	+	+	+	S	+	+	+	+	−	Apr. 20, 1920, bred.
May 21, 1920	P	P	+	+	+	S	+	+	+	+	S	May 5, 1920, three guinea pigs inoculated with milk sediment. When destroyed, June 17, 1920, gave positive agglutination reactions and positive cultural results.
June 5, 1920	P	P	+	+	+	+	+	+	+	+	P	
June 18, 1920	P	P	+	+	+	+	+	+	+	+	P	
July 2, 1920	P	P	+	+	+	S	+	+	+	P	S	May 10, 1920, received 22 cc. fetal stomach contents (case 126).
July 18, 1920	+	+	+	+	+	P	+	+	+	+	−	May 26, 1920, three guinea pigs inoculated with a composite sample of milk. When destroyed, July 27, 1920, positive agglutination reactions and positive cultural results obtained.
July 30, 1920	+	+	+	+	+	S	+	+	+	+	−	
Aug. 13, 1920	+	+	+	+	+	S	+	+	+	+	−	
Aug. 27, 1920	+	+	+	+	+	S	+	+	+	+	−	Dec. 7, 1920, gave birth to a weak calf. Failure experienced in isolation of *Bact. abortus* from placenta.
Sept. 10, 1920	+	+	+	+	+	+	+	+	+	+	−	

TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED COW NO. 410—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Sept. 25, 1920	+	+	+	+	+	+	+	+	+	−	P	Dec. 14, 1920, animal slaughtered. *Bact. abortus* isolated from uterus and milk by cultural methods.
Oct. 7, 1920	+	+	+	+	+	S	+	+	+	+	S	
Oct. 22, 1920	+	+	+	+	+	+	+	+	+	+	S	
Nov. 8, 1920	+	+	+	+	+	+	+	+	+	+	−	
Nov. 27, 1920	+	+	+	+	+	+	+	+	+	+	P	
Dec. 11, 1920	+	+	+	+	+	+	+	+	+	+	−	

VACCINATED COW NO. 416

Feb. 13, 1920	−	−	−	−	−	−	−	−	−	−	−	
Feb. 26, 1920	−	−	−	−	−	−	−	−	−	−	−	Mar. 2, 1920, subcutaneous administration 20 cc. abortion vaccine.
Mar. 13, 1920	+	+	P	S	−	−	P	−	−	−	−	Mar. 20, 1920, four guinea pigs inoculated with milk. Negative cultural results and negative agglutination reactions for *Bact. abortus* when destroyed.
Mar. 27, 1920	+	+	+	+	P	−	+	+	+	−	−	
Apr. 10, 1920	+	+	+	+	+	S	+	+	+	P	−	
Apr. 24, 1920	+	+	+	P	P	−	+	+	+	S	−	Apr. 15, 1920, four guinea pigs inoculated with milk. Negative cultural results and negative agglutination reactions for *Bact. abortus* when destroyed, June 5, 1920.
May 7, 1920	+	+	+	P	S	−	+	+	P	−	−	
May 21, 1920	+	+	+	S	−	−	+	+	−	−	−	
June 5, 1920	+	+	P	S	−	−	+	P	−	−	−	May 5, 1920, three guinea pigs inoculated with milk. Negative cultural results and negative agglutination reactions when destroyed, June 17, 1920.
June 18, 1920	+	P	S	−	−	−	+	−	−	−	−	
July 2, 1920	+	P	S	−	−	−	−	−	−	−	−	May 5, 1920, bred.
July 18, 1920	S	S	−	−	−	−	−	−	−	−	−	May 10, 1920, received 22 cc. fetal stomach contents (case 126).
July 30, 1920	S	−	−	−	−	−	−	−	−	−	−	May 17, 1920, three guinea pigs inoculated with milk; when destroyed gave negative cultural results and negative agglutination reactions.
Aug. 13, 1920	+	+	P	P	−	−	+	+	−	−	−	
Aug. 22, 1920	+	+	+	+	P	S	+	+	+	+	S	July 2, 1920, three guinea pigs inoculated with milk; when destroyed, Sept. 3, 1920, gave negative cultural results and negative agglutination reactions.
Sept. 10, 1920	+	+	+	+	+	P	+	+	+	+	+	
Sept. 25, 1920	+	+	+	+	+	+	+	+	+	+	+	Aug. 22, 1920, three guinea pigs inoculated with milk; when destroyed, Sept. 13, 1920, gave positive agglutination reactions and positive cultural results for *Bact. abortus*.
Oct. 7, 1920	+	+	+	+	+	S	+	+	+	+	−	
Oct. 22, 1920	+	+	+	+	+	+	+	+	+	+	−	
Nov. 8, 1920	+	+	+	+	+	P	+	+	+	+	S	Feb. 19, 1921, expelled a living calf. Three guinea pigs inoculated with an emulsion of placenta when destroyed gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
Nov. 27, 1920	+	+	+	+	+	−	+	+	+	+	−	
Dec. 11, 1920	+	+	+	+	+	−	+	+	+	+	−	
Dec. 29, 1920	+	+	+	P	P	−	+	+	+	P	−	Feb. 28, 1921, *Bact. abortus* isolated from milk by cultural methods.
Jan. 20, 1921	+	+	+	P	S	−	+	+	+	−	−	
Feb. 14, 1921	+	+	+	P	S	−	+	+	+	−	−	
Mar. 3, 1921	+	+	+	P	P	−	+	+	+	−	−	
Mar. 17, 1921	+	+	P	P	P	−	+	+	+	−	−	
Apr. 1, 1921	+	+	P	P	S	−	+	+	P	−	−	

TABLE V.—*Detailed records of individual experimental animals*—Continued

BACTERIN-TREATED HEIFER NO. 423

Date	Serological results: Agglutination							Complement fixation					Remarks
	0.04	0.02	0.01	0.005	0.002	0.001		0.04	0.02	0.01	0.005	0.002	
Nov. 3, 1916	−	−	−	−	−	−		−	−	−	−	−	
Feb. 2, 1917	−	−	−	−	−	−		−	−	−	−	−	Feb. 1, 1917, subcutaneous administration 20 cc. abortion bacterin.
Feb. 8, 1917	+	+	+	S	−	−		+	+	+	+	S	Mar. 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
Feb. 19, 1917	+	+	+	+	−	−		+	+	+	S	−	Mar. 23, 1917, bred.
Mar. 3, 1917	+	+	+	+	−	−		+	+	+	S	−	
Mar. 14, 1917	+	+	−	−	−	−		+	+	+	−	−	
Mar. 22, 1917	+	+	S	−	−	−		+	+	−	−	−	
Apr. 2, 1917	+	P	−	−	−	−		+	+	S	−	−	Apr. 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
Apr. 14, 1917	+	+	P	−	−	−		+	+	+	S	−	May 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
Apr. 23, 1917	P	P	S	−	−	−		+	+	P	−	−	
May 12, 1917	+	+	+	P	S	−		+	+	+	+	−	June 7, 1917, subcutaneous administration 20 cc. abortion bacterin.
June 1, 1917	P	P	P	−	−	−		+	+	P	−	−	June 8, 1917, received 50 cc. fetal-stomach contents (case 21).
June 16, 1917	+	P	S	−	−	−		+	P	−	−	−	
June 29, 1917	+	P	S	S	−	−		+	+	−	−	−	July 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
July 13, 1917	+	P	S	−	−	−		+	+	+	−	−	
July 27, 1917	+	P	S	−	−	−		+	+	P	−	−	Aug. 4, 1917, subcutaneous administration 20 cc. abortion bacterin.
Aug. 11, 1917	+	+	+	P	−	−		+	+	+	P	−	
Aug. 26, 1917	+	+	P	P	−	−		+	+	S	−	−	Sept. 4, 1917, subcutaneous administration 20 cc. abortion bacterin.
Sept. 7, 1917	+	P	S	−	−	−		+	+	+	P	−	
Sept. 21, 1917	+	S	S	−	−	−		+	+	+	−	−	Oct. 5, 1917, subcutaneous administration 20 cc. abortion bacterin.
Oct. 4, 1917	+	−	−	−	−	−		+	+	+	−	−	
Oct. 18, 1917	+	+	P	P	−	−		+	+	+	S	S	
Nov. 1, 1917	+	+	+	+	−	−		+	+	+	+	−	Nov. 3, 1917, subcutaneous administration 20 cc. abortion bacterin.
Nov. 14, 1917	+	+	P	P	−	−		+	+	+	P	−	
Nov. 28, 1917	+	P	S	S	−	−		+	+	+	S	−	Dec. 3, 1917, subcutaneous administration 20 cc. abortion bacterin.
Dec. 13, 1917	+	P	S	−	−	−		+	+	+	−	−	
Jan. 4, 1918	P	S	−	−	−	−		+	+	P	−	−	Dec. 30, 1917, expelled a living calf. 6 guinea pigs inoculated with emulsion of placenta. 3 pigs when destroyed, Mar. 2, 1918, gave negative agglutination reactions and negative cultural results for *Bact. abortus*. 3 pigs which died gave negative cultural results.
Jan. 18, 1918	+	+	+	+	P	P		+	+	+	+	−	
Feb. 2, 1918	+	+	P	P	S	−		+	+	+	+	−	
Feb. 16, 1918	+	+	+	P	−	−		+	+	+	+	−	
Mar. 1, 1918	+	+	P	P	−	−		+	+	+	P	−	
Mar. 16, 1918	+	+	P	P	−	−		+	+	+	+	+	
Mar. 30, 1918	+	P	P	−	−	−		+	+	+	+	P	
Apr. 13, 1918	+	+	+	S	−	−		+	+	+	P	−	
Apr. 29, 1918	+	+	P	P	P	−		+	+	+	P	−	
May 10, 1918	+	P	P	P	−	−		+	+	+	S	−	June 5, 1918, bred.
May 25, 1918	P	P	P	−	−	−		+	+	+	S	−	
June 8, 1918	P	P	S	−	−	−		+	+	+	+	−	
June 21, 1918	P	P	P	S	S	−		+	+	−	−	−	
July 6, 1918	P	P	P	S	−	−		+	+	+	−	−	
July 19, 1918	P	P	S	−	−	−		+	+	+	−	−	
Aug. 1, 1918	P	−	−	−	−	−		+	+	+	−	−	Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
Aug. 16, 1918	S	S	−	−	−	−		+	+	+	−	−	
Aug. 30, 1918	P	P	S	−	−	−		+	+	+	−	−	
Sept. 14, 1918	P	P	P	−	−	−		+	+	+	−	−	
Sept. 28, 1918	+	+	P	P	P	S		+	+	+	+	+	
Oct. 12, 1918	+	+	+	P	P	S		+	+	+	+	+	
Oct. 25, 1918	+	+	+	P	P	S		+	+	+	+	+	
Nov. 8, 1918	+	+	+	S	S	−		+	+	+	+	+	
Nov. 22, 1918	+	+	+	P	S	−		+	+	+	+	+	
Dec. 6, 1918	+	+	P	P	S	−		+	+	+	+	+	
Dec. 20, 1918	+	+	P	P	S	−		+	+	+	+	+	
Jan. 8, 1919	+	+	P	P	P	S		+	+	+	+	+	

TABLE V.—*Detailed records of individual experimental animals*—Continued

BACTERIN-TREATED HEIFER NO. 423—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Jan. 22, 1919	P	+	+	+	P	S	+	+	+	+	+	Jan. 22, 1919, expelled a dead fetus. Failure experienced in isolation of *Bact. abortus* from fetus. 3 guinea pigs inoculated with an emulsion of placenta succumbed to septic infection.
Feb. 7, 1919	P	P	+	+	P	P	+	+	+	+	+	
Feb. 21, 1919	P	P	+	+	P	S	+	+	+	+	+	
Mar. 7, 1919	+	+	+	+	P	S	+	+	+	+	+	Jan. 28, 1919, 4 guinea pigs inoculated with uterine material. When destroyed, Mar. 3, 1919, negative agglutination reactions and negative cultural results for *Bact. abortus* obtained.
Mar. 21, 1919	P	+	+	+	P	S	+	+	+	+	+	

BACTERIN-TREATED HEIFER NO. 401

Nov. 3, 1916	−	−	−	−	−	−	−	−	−	−	−	Aug. 8, 1917, bred.
Jan. 7, 1918	−	−	−	−	−	−	−	−	−	−	−	
Jan. 18, 1918	+	P	P	S	−	−	+	+	−	−	−	Dec. 17, 1917, subcutaneous administration 20 cc. abortion bacterin.
Feb. 2, 1918	+	+	S	S	−	−	+	+	−	−	−	Jan. 2, 1918, subcutaneous administration 20 cc. abortion bacterin.
Feb. 16, 1918	P	S	−	−	−	−	+	+	−	−	−	Jan. 5, 1918, received 30 cc. fetal-stomach contents (case 54).
Mar. 1, 1918	+	+	−	−	−	−	+	+	−	−	−	Jan. 22, 1918, subcutaneous administration 20 cc. abortion bacterin.
Mar. 16, 1918	+	+	P	−	−	−	+	+	+	−	−	Feb. 2, 1918, subcutaneous administration 20 cc. abortion bacterin.
Mar. 30, 1918	P	S	S	−	−	−	+	+	S	−	−	Mar. 4, 1918, subcutaneous administration 20 cc. abortion bacterin.
Apr. 13, 1918	P	S	S	−	−	−	+	S	−	−	−	Mar. 25, 1918, subcutaneous administration 20 cc. abortion bacterin.
Apr. 29, 1918	+	+	P	P	S	−	+	S	−	−	−	
May 10, 1918	+	P	P	−	−	−	+	+	P	−	−	Apr. 15, 1918, subcutaneous administration 20 cc. abortion bacterin.
May 25, 1918	+	+	+	P	−	−	+	+	+	+	−	Mar. 8, 1918, expelled a living calf. 4 guinea pigs inoculated with emulsion of placenta. 2 pigs, destroyed July 24, 1918, gave negative agglutination reactions and negative cultural results for *Bact. abortus*. Two pigs not cultured.
June 8, 1918	+	+	+	P	−	−	+	+	+	+	−	
June 21, 1918	+	+	+	P	−	−	+	+	+	−	−	
July 6, 1918	+	+	+	P	S	−	+	+	P	−	−	
July 19, 1918	+	+	P	P	P	−	+	+	P	−	−	
Aug. 1, 1918	+	+	S	−	−	−	+	+	−	−	−	
Aug. 16, 1918	+	+	P	P	−	−	+	+	+	−	−	
Aug. 30, 1918	+	+	+	P	−	−	+	+	+	−	−	
Sept. 14, 1918	+	+	+	+	−	−	+	+	+	−	−	Sept. 23, 1918, bred.
Sept. 28, 1918	+	+	P	S	−	−	+	+	P	−	−	Oct. 14, 1918, bred.
Oct. 12, 1918	+	+	P	S	−	−	+	+	−	−	−	
Oct. 26, 1918	+	P	P	S	−	−	+	+	−	−	−	
Nov. 8, 1918	+	P	P	P	−	−	+	+	−	−	−	Nov. 13, 1918, received 20 cc. fetal-stomach contents (case 74).
Nov. 22, 1918	P	P	S	S	−	−	+	P	−	−	−	
Dec. 6, 1918	P	P	S	−	−	−	+	S	−	−	−	
Dec. 20, 1918	P	P	P	−	−	−	+	S	−	−	−	
Jan. 8, 1919	+	P	P	S	−	−	+	+	−	−	−	
Jan. 22, 1919	+	+	P	P	S	−	+	+	+	−	−	
Feb. 7, 1919	+	+	P	P	−	−	+	+	+	−	−	
Feb. 21, 1919	+	+	+	P	S	−	+	+	+	−	−	
Mar. 7, 1919	+	+	+	+	P	−	+	+	+	+	−	
Mar. 21, 1919	+	+	+	+	+	+	+	+	+	+	P	
Apr. 4, 1919	+	+	+	+	+	+	+	+	+	+	+	
Apr. 19, 1919	+	+	+	+	+	+	+	+	+	+	+	

TABLE V.—*Detailed records of individual experimental animals*—Continued

BACTERIN-TREATED HEIFER NO. 401—Continued

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
May 3, 1919	+	+	+	+	+	+	+	+	+	+	+	May 19, 1919, 4 guinea pigs inoculated with a sample of milk; when destroyed, June 30, 1919, gave positive agglutination reactions and positive cultural results for *Bact. abortus*.
May 17, 1919	+	+	+	+	+	+	+	+	+	+	+	
May 29, 1919	+	+	+	+	+	+	+	+	+	+	+	
June 14, 1919	+	+	+	+	+	+	+	+	+	+	+	
June 28, 1919	+	+	+	+	+	+	+	+	+	+	+	July 16, 1919, 3 guinea pigs inoculated with vaginal mucus. When destroyed, Sept. 9, 1919, negative agglutination reactions and negative cultural results obtained.
July 10, 1919	+	+	+	+	+	+	+	+	+	+	+	
July 25, 1919	+	+	+	+	+	+	+	+	+	+	+	
Aug. 9, 1919	+	+	+	+	+	+	+	+	+	+	+	
Aug. 23, 1919	+	+	+	+	+	+	+	+	+	+	+	July 19, 1919, expelled a living calf. 4 guinea pigs inoculated with emulsion of placenta. Pigs died during following week. July 23, 1919, 4 guinea pigs inoculated with uterine material. When destroyed, Sept. 9, 1919, negative agglutination reactions and negative cultural results obtained.

BACTERIN-TREATED HEIFER No. 404

Nov. 3, 1916	−	−	−	−	−	−	−	−	−	−	−	
Feb. 2, 1917	−	−	−	−	−	−	−	−	−	−	−	Feb. 1, 1917, subcutaneous administration 20 cc. abortion bacteria.
Feb. 8, 1917	P	P	−	−	−	−	P	−	−	−	−	
Feb. 19, 1917	+	+	P	S	−	−	+	+	−	−	−	Mar. 1, 1917, subcutaneous administration 20 cc. abortion bacterin.
Mar. 3, 1917	+	+	P	−	−	−	+	+	−	−	−	
Mar. 14, 1917	+	P	P	S	−	−	+	P	S	−	−	
Mar. 22, 1917	+	P	S	−	−	−	+	−	−	−	−	Apr. 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
Apr. 3, 1917	+	P	−	−	−	−	+	−	−	−	−	
Apr. 14, 1917	+	+	P	−	−	−	+	+	+	−	−	
Apr. 23, 1917	+	P	P	−	−	−	+	+	−	−	−	May 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
May 12, 1917	+	+	+	S	−	−	+	+	+	−	−	
June 1, 1917	+	+	+	P	−	−	+	+	S	S	−	May 24, 1917, bred.
June 16, 1917	+	P	S	−	−	−	+	+	−	−	−	June 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
June 29, 1917	+	P	−	−	−	−	+	P	−	−	−	June 7, 1917, received 50 cc. fetal stomach contents (case 21).
July 13, 1917	+	P	P	−	−	−	+	+	−	−	−	July 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
July 27, 1917	+	P	S	−	−	−	+	+	−	−	−	
Aug. 11, 1917	+	P	P	S	−	−	+	+	+	−	−	Aug. 4, 1917, subcutaneous administration 20 cc. abortion bacterin.
Aug. 26, 1917	+	+	P	S	−	−	+	+	+	−	−	
Sept. 7, 1917	+	P	P	−	−	−	+	+	+	−	−	Sept. 4, 1917, subcutaneous administration 20 cc. abortion bacterin.
Sept. 21, 1917	+	P	S	−	−	−	+	+	−	−	−	
Oct. 4, 1917	+	+	S	−	−	−	+	+	S	−	−	Oct. 5, 1917, subcutaneous administration 20 cc. abortion bacterin.
Oct. 18, 1917	+	+	+	+	−	−	+	+	+	+	P	
Nov. 1, 1917	+	+	+	+	−	−	+	+	+	+	+	Nov. 2, 1917, expelled a dead fetus. *Bact. abortus* isolated therefrom.
Nov. 14, 1917	+	+	+	+	P	P	+	+	+	+	+	
Nov. 28, 1917	+	+	+	P	P	S	+	+	+	+	+	
Dec. 13, 1917	+	+	+	+	P	−	+	+	+	+	+	

TABLE V.—*Detailed records of individual experimental animals*—Continued

BACTERIN-TREATED HEIFER No. 464

Date	Serological results: Agglutination 0.40	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Nov. 28, 1916	−	−	−	−	−	−	−	−	−	−	−	
Oct. 6, 1917	−	−	−	−	−	−	−	−	−	−	−	June 25, 1917, bred.
Oct. 18, 1917	S	−	−	−	−	−	−	−	−	−	−	Oct. 5, 1917, subcutaneous administration 10 cc. abortion bacterin.
Nov. 1, 1917	P	S	−	−	−	−	S	−	−	−	−	Oct. 20, 1917, subcutaneous administration 10 cc. abortion bacterin.
Nov. 14, 1917	P	S	−	−	−	−	S	S	−	−	−	Nov. 2, 1917, received 5 cc. fetal-stomach contents (case 42).
Nov. 28, 1917	P	S	−	−	−	−	S	S	−	−	−	Nov. 2, 1917, subcutaneous administration 10 cc. abortion bacterin.
Dec. 13, 1917	P	P	S	−	−	−	+	S	−	−	−	Nov. 16, 1917, subcutaneous administration 10 cc. abortion bacterin.
Jan. 4, 1918	+	+	P	P	−	−	+	P	S	−	−	Dec. 3, 1917, subcutaneous administration 10 cc. abortion bacterin.
Jan. 18, 1918	+	+	+	P	−	−	+	+	+	−	−	Dec. 18, 1917, subcutaneous administration 10 cc. abortion bacterin.
Feb. 2, 1918	+	+	+	P	−	−	+	+	+	−	−	Jan. 2, 1918, subcutaneous administration 10 cc. abortion bacterin.
Feb. 16, 1918	+	+	+	+	+	+	+	+	+	+	+	Jan. 31, 1918, subcutaneous administration 10 cc. abortion bacterin.
Mar. 1, 1918	+	+	+	+	+	+	+	+	+	+	+	Feb. 14, 1918, subcutaneous administration 10 cc. abortion bacterin.
Mar. 16, 1918	+	+	+	+	+	P	+	+	+	+	+	Feb. 18, 1918, expelled a dead fetus. *Bact. abortus* isolated therefrom.
Mar. 30, 1918	+	+	+	P	P	P	+	+	+	+	+	

CONTROL HEIFER No. 446

Nov. 28, 1916	−	−	−	−	−	−	−	−	−	−	−	May 4, 1917, bred.
June 16, 1917	−	−	−	−	−	−	−	−	−	−	−	June 8, 1917, received 50 cc. fetal-stomach contents (case 21).
June 29, 1917	−	−	−	−	−	−	−	−	−	−	−	
July 13, 1917	−	−	−	−	−	−	−	−	−	−	−	
July 27, 1917	−	−	−	−	−	−	−	−	−	−	−	
Aug. 11, 1917	−	−	−	−	−	−	−	−	−	−	−	
Aug. 26, 1917	−	−	−	−	−	−	−	−	−	−	−	
Sept. 7, 1917	−	−	−	−	−	−	−	−	−	−	−	
Sept. 21, 1917	−	−	−	−	−	−	−	−	−	−	−	
Oct. 4, 1917	−	−	−	−	−	−	−	−	−	−	−	
Oct. 18, 1917	+	+	−	−	−	−	+	P	−	−	−	
Nov. 1, 1917	+	+	+	S	−	−	+	+	P	−	−	
Nov. 14, 1917	+	+	+	+	−	−	+	+	+	−	−	Nov. 2, 1917, expelled a dead fetus. *Bact. abortus* isolated therefrom by cultural methods.
Nov. 28, 1917	+	+	+	+	+	−	+	+	+	+	−	
Dec. 13, 1917	+	+	+	+	+	S	+	+	+	+	+	

CONTROL HEIFER No. 422

Nov. 3, 1916	−	−	−	−	−	−	−	−	−	−	−	May 4, 1917, bred.
June 16, 1917	−	−	−	−	−	−	−	−	−	−	−	June 8, 1917, received 50 cc. fetal-stomach contents (case 21).
June 29, 1917	−	−	−	−	−	−	−	−	−	−	−	
July 13, 1917	+	P	S	−	−	−	−	−	−	−	−	
July 27, 1917	P	P	S	−	−	−	−	−	−	−	−	
Aug. 11, 1917	+	+	S	−	−	−	S	−	−	−	−	
Aug. 26, 1917	P	S	−	−	−	−	+	P	−	−	−	
Sept. 7, 1917	P	−	−	−	−	−	P	S	−	−	−	
Sept. 21, 1917	S	−	−	−	−	−	+	+	−	−	−	
Oct. 4, 1917	+	−	−	−	−	−	+	S	−	−	−	
Oct. 18, 1917	+	+	P	S	−	−	+	+	+	−	−	Oct. 23, 1917, expelled a dead fetus *Bact. abortus* isolated therefrom by cultural methods.
Nov. 8, 1917	+	+	+	+	−	−	+	+	+	+	+	
Nov. 21, 1917	+	+	+	+	P	−	+	+	+	+	+	
Dec. 7, 1917	+	+	+	+	−	−	+	+	+	+	+	

TABLE V.—*Detailed records of individual experimental animals*—Continued

CONTROL HEIFER No. 460

Date	Serological results: Agglutination 0.40	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Nov. 28, 1916	−	−	−	−	−	−	−	−	−	−	−	
June 16, 1917	−	−	−	−	−	−	−	−	−	−	−	June 16, 1917, bred.
Nov. 2, 1917	−	−	−	−	−	−	−	−	−	−	−	Nov. 2, 1917, received 5 cc. fetal-stomach contents (case 42).
Nov. 14, 1917	−	−	−	−	−	−	−	−	−	−	−	
Nov. 28, 1917	−	−	−	−	−	−	−	−	−	−	−	
Dec. 13, 1917	−	−	−	−	−	−	−	−	−	−	−	
Jan. 4, 1918	−	−	−	−	−	−	−	−	−	−	−	
Jan. 18, 1918	−	−	−	−	−	−	−	−	−	−	−	
Feb. 2, 1918	−	−	−	−	−	−	−	−	−	−	−	
Feb. 16, 1918	−	−	−	−	−	−	−	−	−	−	−	
Mar. 1, 1918	S	S	−	−	−	−	−	−	−	−	−	Mar. 3, 1918, expelled a dead fetus. *Bact. abortus* isolated therefrom by cultural methods.
Mar. 16, 1918	+	+	+	+	P	S	+	+	+	+	+	
Mar. 30, 1918	+	+	+	+	P	S	+	+	+	+	+	
Apr. 13, 1918	+	+	+	+	+	+	+	+	+	+	+	
Apr. 29, 1918	+	+	+	+	+	P	+	+	+	+	+	
May 10, 1918	+	+	+	+	+	P	+	+	+	+	+	

CONTROL HEIFER No. 450

Nov. 28, 1916	−	−	−	−	−	−	−	−	−	−	−	Aug. 16, 1917, bred.
Jan. 4, 1918	−	−	−	−	−	−	−	−	−	−	−	Dec. 13, 1917, received 30 cc. fetal-stomach contents (case 54).
Feb. 2, 1918	P	S	−	−	−	−	−	−	−	−	−	
Feb. 16, 1918	+	P	S	S	−	−	+	+	P	−	−	
Mar. 1, 1918	+	P	P	−	−	−	+	+	S	−	−	
Mar. 16, 1918	+	+	P	S	−	−	+	+	+	S	−	
Mar. 30, 1918	+	+	+	−	−	−	+	+	+	+	−	
Apr. 13, 1918	+	+	+	P	S	−	+	+	+	+	+	Apr. 14, 1918, expelled a dead fetus. *Bact. abortus* isolated therefrom by cultural methods.
Apr. 29, 1918	+	+	+	+	P	P	+	+	+	+	+	
May 10, 1918	+	+	+	+	P	S	+	+	+	+	+	
May 25, 1918	+	+	+	+	P	P	+	+	+	+	+	
June 8, 1918	+	+	+	+	P	P	+	+	+	+	+	

CONTROL HEIFER No. 413

June 6, 1917	−	−	−	−	−	−	−	−	−	−	−	May 9, 1917, bred.
June 29, 1917	−	−	−	−	−	−	−	−	−	−	−	June 8, 1917, received 50 cc. fetal-stomach contents (case 21).
July 13, 1917	−	−	−	−	−	−	−	−	−	−	−	
July 27, 1917	−	−	−	−	−	−	−	−	−	−	−	
Aug. 11, 1917	−	−	−	−	−	−	−	−	−	−	−	
Aug. 26, 1917	−	−	−	−	−	−	−	−	−	−	−	
Sept. 7, 1917	−	−	−	−	−	−	−	−	−	−	−	
Sept. 21, 1917	−	−	−	−	−	−	−	−	−	−	−	
Oct. 4, 1917	−	−	−	−	−	−	−	−	−	−	−	
Oct. 18, 1917	P	−	−	−	−	−	−	−	−	−	−	
Nov. 1, 1917	+	+	P	−	−	−	−	−	−	−	−	
Nov. 14, 1917	+	+	P	−	−	−	+	+	+	−	−	
Dec. 13, 1917	+	+	+	+	P	−	+	+	+	+	+	Dec. 1, 1917, expelled a dead fetus. *Bact. abortus* isolated therefrom by cultural methods.
Jan. 4, 1918	+	+	+	+	P	−	+	+	+	+	+	
Jan. 18, 1918	+	+	+	+	+	+	+	+	+	+	+	
Feb. 2, 1918	+	+	+	+	+	+	+	+	+	+	+	

TABLE V.—*Detailed records of individual experimental animals*—Continued

CONTROL HEIFER NO. 430

Date	Serological results: Agglutination 0.04	0.02	0.01	0.005	0.002	Complement fixation 0.001	0.04	0.02	0.01	0.005	0.002	Remarks
Feb. 28, 1918	−	−	−	−	−	−	−	−	−	−	−	Jab. 7, 1919, bred.
July 11, 1918	−	−	−	−	−	−	−	−	−	−	−	Feb. 24, 1919, received 20 cc. fetal-stomach contents (case 60).
Mar. 7, 1919	−	−	−	−	−	−	−	−	−	−	−	Mar. 19, 1919, received 25 cc. fetal-stomach contents (case 88).
Mar. 21, 1919	−	−	−	−	−	−	−	−	−	−	−	
June 26, 1919	+	+	+	+	+	+	+	+	+	+	+	
July 10, 1919	+	+	+	+	+	+	+	+	+	+	+	
July 25, 1919	+	+	+	+	+	+	+	+	+	+	+	Aug. 13, 1919, expelled a dead fetus. *Bact. abortus* isolated from fetus by cultural methods.
Aug. 23, 1919	+	+	+	+	+	+	+	+	+	+	+	
Sept. 6, 1919	+	+	+	+	+	+	+	+	+	+	+	Oct. 6, 1919, slaughtered. *Bact abortus* isolated from udder by cultural methods. Failure experienced in isolation of *Bact. abortus* from uterus culturally.
Sept. 19, 1919	+	+	+	+	+	+	+	+	+	+	+	

CONTROL COW NO. 470

Dec. 20, 1918	−	−	−	−	−	−	−	−	−	−	−	
Jan. 8, 1919	−	−	−	−	−	−	−	−	−	−	−	
Jan. 22, 1919	−	−	−	−	−	−	−	−	−	−	−	
Feb. 7, 1919	−	−	−	−	−	−	−	−	−	−	−	
Feb. 21, 1919	−	−	−	−	−	−	−	−	−	−	−	Feb. 24, 1919, bred.
Mar. 7, 1919	−	−	−	−	−	−	−	−	−	−	−	
Mar. 21, 1919	−	−	−	−	−	−	−	−	−	−	−	
Apr. 4, 1919	−	−	−	−	−	−	−	−	−	−	−	
Apr. 19, 1919	−	−	−	−	−	−	−	−	−	−	−	
May 3, 1919	−	−	−	−	−	−	−	−	−	−	−	
May 17, 1919	−	−	−	−	−	−	−	−	−	−	−	
May 30, 1919	−	−	−	−	−	−	−	−	−	−	−	May 24, 1919, received 50 cc. fetal-stomach contents (case 92).
June 14, 1919	P	P	−	−	−	−	−	−	−	−	−	
June 25, 1919	P	S	−	−	−	−	−	−	−	−	−	Oct. 11, 1919, three guinea pigs inoculated with a sample of milk; when destroyed, Nov. 26, 1919, gave negative agglutination reactions and negative cultural results for *Bact. abortus*.
July 10, 1919	P	S	−	−	−	−	−	−	−	−	−	
July 25, 1919	P	−	−	−	−	−	−	−	−	−	−	
Aug. 9, 1919	P	−	−	−	−	−	−	−	−	−	−	
Aug. 23, 1919	P	S	−	−	−	−	−	−	−	−	−	Oct. 22, 1919, *Bact. abortus* isolated from milk by cultural methods and guinea-pig inoculations.
Sept. 6, 1919	+	+	P	P	P	−	+	+	+	+	P	
Sept. 19, 1919	+	+	+	+	P	P	+	+	+	+	+	
Oct. 4, 1919	+	+	+	+	P	P	+	+	+	+	+	Nov. 17, 1919, expelled a weak calf. Placenta showed excessive necrosis. Three guinea pigs inoculated with an emulsion of placenta, when destroyed, Jan. 7, 1920, gave positive agglutination reactions and positive cultural results for *Bact. abortus*.
Oct. 17, 1919	+	+	+	+	P	P	+	+	+	+	+	
Oct. 31, 1919	+	+	+	+	P	P	+	+	+	+	+	
Nov. 14, 1919	+	+	+	P	P	P	+	+	+	+	+	
Nov. 29, 1919	+	+	+	P	S	S	+	+	+	+	+	
Dec. 13, 1919	+	+	+	P	S	S	+	+	+	+	+	
Jan. 3, 1920	+	+	+	P	S	S	+	+	+	+	+	

TABLE V.—*Detailed records of individual experimental animals*—Continued

CONTROL COW NO. 453

Date	Serological results: Agglutination 0.40	0.02	0.01	0.005	0.002	0.001	Complement fixation 0.04	0.02	0.01	0.005	0.002	Remarks
Feb. 28, 1920	−	−	−	−	−	−	−	−	−	−	−	Dec. 18, 1919, bred.
Mar. 13, 1920	−	−	−	−	−	−	−	−	−	−	−	
Mar. 27, 1920	−	−	−	−	−	−	−	−	−	−	−	
Apr. 10, 1920	−	−	−	−	−	−	−	−	−	−	−	
Apr. 24, 1920	−	−	−	−	−	−	−	−	−	−	−	
May 7, 1920	−	−	−	−	−	−	−	−	−	−	−	May 10, 1920, received 22 cc. fetal stomach contents (case 126).
May 21, 1920	−	−	−	−	−	−	−	−	−	−	−	
June 5, 1920	+	P	S	−	−	−	P	−	−	−	−	June 22, 1920, expelled a dead fetus. Placenta necrotic. *Bact. abortus* isolated from placenta by cultural methods and guinea-pig inoculations.
June 18, 1920	+	+	+	+	P	S	+	P	−	−	−	
July 2, 1920	+	+	+	+	P	P	+	+	+	P	−	
July 18, 1920	P	+	+	P	P	P	+	+	+	+	−	
July 30, 1920	P	+	+	+	P	P	+	+	+	+	P	July 2, 1920, three guinea pigs inoculated with milk: when destroyed, Sept. 3, 1920, gave positive agglutination reactions and positive cultural results for *Bact. abortus*.
Aug. 13, 1920	P	+	+	+	+	P	+	+	+	+	S	
Aug. 27, 1920	P	+	+	+	+	P	+	+	+	+	+	
Sept. 10, 1920	P	+	+	+	+	P	+	+	+	+	+	
Sept. 21, 1920	P	+	+	+	+	P	+	+	+	+	+	

THE RHIZOCTONIA BROWN ROT AND OTHER FRUIT ROTS OF STRAWBERRIES[1]

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INTRODUCTION

The fruit rots of strawberries have received much less attention than the importance of the crop and the great losses from decay would seem to justify. A number of fungi are known which attack strawberries and it is to be expected that as investigation continues there will be discovered numerous other species which in certain localities or under some conditions are capable of causing serious fruit rots. Very recently Rose [2] has published the results of an extensive study of a fruit rot of strawberries found to be destructive in the Arkansas and Tennessee regions and which he calls "leather rot." In the present paper the writers report a new or little known field rot which is very important in Florida. Since both these diseases produce a brown discoloration, and at least two other so-called "brown rots" of strawberries are already known, it seems necessary to briefly discuss at this time the distinguishing characters of these rots.

THE BROWN RHIZOCTONIA ROT

Each season since 1920 as strawberries have come into the Washington market from Florida the writers have observed a peculiar rot on many of the berries which appeared to be perfectly sound on one side. Some of these berries were deformed as though one side had failed to fill out properly. This part of the berry was commonly affected with a somewhat dry brown rot which often had a blackish color, due chiefly to the adhering black sandy soil. This suggested that the rot might be due to some soil organism.

Study of the rots in Florida strawberry fields, especially in the vicinity of Plant City in 1923, and Kissimmee in 1924, shows that while Botrytis appears in epidemics, suddenly and with very destructive effects during or soon after brief periods of rainy weather, the rot just mentioned as found in the Washington markets develops much more regularly in the fields and makes it necessary to cull out large numbers of berries during picking and packing. As this disease is almost always present, yet rarely if ever epidemic, it attracts relatively little attention among growers. Continued observation has convinced the writers, however, that it is a constant source of loss to strawberry growers in central Florida and, until a remedy is discovered, will remain a handicap to the industry. Careful observation has convinced the writers that Rhizoctonia caused at least half the loss from field rots in this region during the season of 1923–24.

The characters of this rot are so pronounced that it can be distinguished readily from the other so-called brown rots of strawberry. Affected berries are generally one-sided, and show a hard brown rot, to which often adhere quantities of sandy soil. Decay regularly starts on the under side of the berry where it comes in contact with the soil, and a small amount of soil will usually be found clinging to the decayed spot even in dry weather. If the soil is of the black

[1] Received for publication Feb. 25, 1924

[2] ROSE, D. H. LEATHER ROT OF STRAWBERRIES. Jour. Agr. Research, 28: 357-376, illus. 1924.

sandy type common in low places in many Florida strawberry fields, the surface of the rotting area will of course appear blackish (Pl. 1, A, C). If little or no soil clings to the berry, the true dark brown color of the rot will be evident (Pl. 1, D). The rot usually starts before the berry begins to turn red, sometimes even before the fruit is a third grown (Pl. 1, E). In this case the discolored side will be of a lighter brown, and only a little soil will adhere. An early attack on the berry will result in a deformed fruit, but as the rot progresses rather slowly, the upper side of the berry develops normally and ripens without showing rot, so that one can not tell that the berry is diseased until it is turned over. As a result many such berries are picked unintentionally and only extreme care will prevent their being overlooked by packers.

Sections of the rotting berry show that there is a definite line of demarcation in the pulp indicating clearly how far the disease has spread. Where healthy and diseased tissues meet, the pulp is only slightly faded and brownish; otherwise the diseased area stands out sharply on account of its rich brown color. The rotted part later becomes dried out or mummified (Pl. 1, B). By using a hand lens one is able to see masses of hyphae which bind the particles of soil together and hold the dirt to the surface of the berry. It will be shown that these soil hyphae belong to the Rhizoctonia which is causing the rot. In a free-hand section of the browning pulp examined under the microscope can be seen vast numbers of hyphae crowding in between the cells in the more recently invaded regions (Pl. 2, *B*, *a*), while in those parts of the berry where the fungus has completed its work the cells are crushed and distorted and the spaces are packed with felty masses of mycelium. If the affected part of the berry is cut away the remainder will be found to be perfectly edible (Pl. 1, C), Such rot fungi as Botrytis and Rhizopus grow through the berry and usually completely destroy it once they have gained a foothold. The appearance of berries attacked by the hard rot is such that they will be discarded wherever found. The rot develops so slowly that it would not be communicated rapidly to other berries under refrigeration in transit.

Whether the fungus, as it advances between the cells, attacks only the middle lamellae, as is so frequently reported for other intercellular fungi, is uncertain. At first the cells of the invaded tissues actually become hypertrophied, consequently the intercellular hyphae are put under pressure and are compelled to force back the elastic cell walls as they advance (Pl. 2, B, *c*). Certainly water is not withdrawn from the pulp cells in mass, resulting either in a wet rot such as is produced by Rhizopus or in a separation or falling apart of host cells, such as follows the work of Pezizella. Water seems to be lost more by a drying out process. Cells in the decaying tissues gradually collapse after the period of swelling and the hyphae crowd in, becoming more and more intracellular. The feeding of the hyphae in the middle lamellae may be the cause of a sort of mucilaginous disorganization which is manifested by the presence of quantities of intercellular stainable substance which so often surrounds the hyphae. This is more noticeable as the disease progresses. Striae or laminations in the adjacent walls are then more distinct. The intercellular substance and the peculiar manner in which the mucilaginous layers are split apart are brought out in Plate 2, figure B, *b*. The contents of the unstained hyphae are foamy or vacuolate.

MYCELIAL CHARACTERS

The mycelium of the fungus is comprised of septate branching hyphae of fairly uniform diameter, though where a branch grows out the diameter is somewhat less. In some cases hyphae are very coarse for short distances and branches are very irregular with blunt ends. The cells usually contain more than one

nucleus, sometimes as many as five; two or three being the common numbers. The nuclear wall is not very distinct in stained preparations but the large nucleole is always plainly visible, surrounded by a clear place in the cytoplasm (Pl. 2, A, *b*).

ARTIFICIAL INOCULATIONS ON STRAWBERRIES

Strawberries growing in pots in the greenhouse at the Arlington experiment farm, Virginia, were readily inoculated by laying pieces of agar bearing mycelium on the soil beneath half-grown berries. In every case the berries developed the characteristic rot within three or four days. The fungus penetrated the soil at the point attacked so that when the berry was removed some of the soil was attached to it. The fungus was recovered from such diseased berries by transfer of pulp from points well below the surface. Greenish berries from the Washington market have also been inoculated in damp chambers by placing bits of agar covered with hyphae from test-tube cultures on the bottom of damp chambers and then laying an apparently sound berry on the piece of agar. The brown rot developed in every case within two days (Pl. 1, G). Since the epidermis of a strawberry is so liable to rupture from all sorts of causes it is not certain that the fungus actually penetrated the unbroken epidermis.

Sections of berries artificially inoculated show that the hyphae crowd in between the akene and that part of the receptacle in which the akene is partly sunken. It may be that the fungus finds a film of moisture in this region where the walls of the epidermis are also easily penetrated. The most noticeable effect upon the host tissue is a swelling and clearing of the cells between which the hyphae are pushing. The cell contents take very little stain. The fungus apparently does not seek out any particular part of the berry. Hyphae are found in every tissue in the region attacked. The sieve tubes or long conducting cells of the vascular bundles leading to the "seeds" are pushed aside and hyphae crowd in between them or run along between these cells in all directions (Pl. 3, A). Hyphae also pack in between the small cells of the pulp between the akenes and crowd up close to the cells of the sclerotized and thickened cell walls (Pl. 3, B). No living tissue appears to be immune. If nuclei were present in the diseased pulp cells examined, they did not take the stain. The nuclei of the fungus, however, appeared very distinctly (Pl. 2, A, *b;* B).

THE TAN BROWN ROT OF STRAWBERRIES

An excellent description of the Pezizella brown rot of strawberries was published by Stevens and Peterson[3] in connection with their account of the organism causing the disease. They described this fungus as *Patellina fragariae* although it had already been described under a great many different names.[4] The region attacked by the fungus is marked on green berries by the appearance of small sunken spots. These spots are tan colored, slightly sunken, and enlarge only slowly. When a ripe berry is attacked, the rot spreads much more rapidly but the color of the spot is about the same (Pl. 1, H). The fungus grows into the pulp of the berry so that the rotted portion is deeper than its diameter. The core of the region attacked is consumed and the host tissue is replaced by mycelium so that the decayed portion finally presents a spongy dry texture. The host tissue immediately surrounding the diseased area, although devoid of mycelium, becomes soft and wet and its cells separate from each other. As the core of the rotten spot is very tenacious it can easliy be removed intact. This

[3] STEVENS, F. L., and PETERSON, A. SOME NEW STRAWBERRY FUNGI. Phytopathology. 6: 258-267, illus. 1916.

[4] SHEAR, C. L., and DODGE, B. O. THE LIFE HISTORY AND IDENTITY OF "PATELLINA FRAGARIAE," "LEPTOTHYRIUM MACROTHECIUM" AND "PEZIZA OENOTHERAE," Mycologia, 13: 135-170. pl. 8-10. 1921.

characteristic serves for the ready identification of this rot. In addition it is often possible to see with the aid of a hand lens the little "sporodochia" of the Hainesia (Patellina) stage of the fungus on the surface of the spot. Teased and microtome sections show, according to Stevens and Peterson, that the host cells are filled with hyphae.

THE LEATHER ROT OF STRAWBERRIES

The leather rot, as Rose[5] calls the disease, is caused by a Phytophthora very similar to *P. cactorum* and is characterized by a definite though slight softening of the affected tissues and by internal discoloration. It is a typical field rot attacking berries in all stages of growth, and is most common following rainy periods, a characteristic which results in the name "water soak" often used by growers. The results of Rose's inoculation experiments did not lead him to conclude that the hyphae of this Phytophthora can penetrate the unbroken strawberry epidermis. In the field, however, berries touching the ground were first affected on the under side, suggesting that the fungus may be a true soil organism, and that it may be able to penetrate the unbroken epidermis under favorable circumstances.

Green fruit becomes brown where attacked, and fruit that has turned red before the fungus gains entrance presents a series of color changes as the disease progresses. At the center of the spot the color becomes yellow to light brown, spreading out from here the color changes to purple and then to the natural red as sound tissue is approached (Pl. 1, J). The most characteristic feature of the rot is shown in sections of the fruit. The vascular tissue becomes markedly discolored, being a much deeper brown than the affected pulp (Pl. 1, K). In the early stages of the disease vascular browning may be the only symptom. There is no clear line of demarcation between sound and diseased tissue, such as is characteristic of the hard brown rot. Neither can the diseased tissue be scooped out as it can be in the case of the Pezizella rot. One striking characteristic of leather rot is that even slightly rotten berries are bitter to the taste.

THE BOTRYTIS BROWN ROT OF STRAWBERRIES

Strawberries attacked by Botrytis present symptoms which in the early stages of the disease are quite different from those shown in the later stages. As the hyphae penetrate into the pulp it loses its natural color and turns light brown. Later the color becomes somewhat darker (Pl. 1, I). The rotting pulp is at first rather soft, almost watery, though never leaky. This stage is soon superseded by a drying out so that the berry becomes firm. After the disease has run its course, the berry is found to be hard and dry,[6] which results in the use of the term "dry rot" among growers. The tendency, however, to make use of color characters describing rots will always prevail to a certain extent, so that shippers and inspectors no doubt will continue to use the term brown rot for this and similar diseases.

As soon as aerial hyphae with their crop of conidia develop, the surface of the berry presents a characteristic appearance which is referred to as "gray mold." Sections of immature or green berries recently affected with Botrytis rot do not show a distinct line of demarcation between healthy and diseased tissue. The diseased pulp is of a darker brown near the surface. The color shades off to a lighter brown and ends with the natural color of the healthy fruit in parts not invaded by the mycelium. Stevens [7] who investigated the host parasite relation

[5] ROSE, D. H. LEATHER ROT OF STRAWBERRIES. Jour. Agr. Research, 28: 1924.

[6] STEVENS, NEIL E., and WILCOX, R. B. FURTHER STUDIES OF THE ROTS OF STRAWBERRY FRUITS. U. S. Dept. Agr. Bul. 686, 14 p. 1918. Literature cited, p. 14.

[7] STEVENS, NEIL E. PATHOLOGICAL HISTOLOGY OF STRAWBERRIES AFFECTED BY SPECIES OF BOTRYTIS AND RHIZOPUS. Jour. Agr. Research 6: 361-366, pl. 49-50. 1916.

in connection with this disease found that the hyphae penetrate the cell walls readily and dissolve the middle lamellae. The hyphae were found to grow between the cells for some distance, then to penetrate the cell walls becoming intracellular. All parts of the berry are subject to attack by the fungus, which if allowed to develop does not stop at localized infections. It is evident from the way the cytoplasm shrinks away from the cell walls and becomes disorganized that the host cells are killed quickly. Water is so slowly and gradually withdrawn from the host cells that it evaporates from the surface of the berry and no leak is caused, in spite of the fact that hyphae penetrate pulp or storage cells everywhere. The rotting berry thus becomes dried and mummified.

COMPARISON OF THE FOUR BROWN ROTS

The two field rots most likely to be confused with the hard brown rot are those caused by Botrytis and Pezizella, although each has sufficiently distinctive characters to make indentification reasonably certain. The Botrytis rot is a lighter brown and more watery at the beginning. That caused by Pezizella forms a pocket in the sticky scablike rotted portion. In only one case out of 126 cultures made by direct transfer of pulp from berries selected in the field as affected with hard brown rot was Botrytis obtained as a result of faulty diagnosis. The hard rot caused by the Rhizoctonia under discussion in this paper is easily distinguished from the tough dry rot due to Botrytis by the clear line of demarcation between healthy and diseased tissue characteristic of the Rhizoctonia rot.

The Rhizoctonia hard brown rot and the Pezizella soft brown rot are alike in that in both there is a distinct line separating the diseased from the healthy tissue. Not infrequently berries infected with the Rhizoctonia when shipped arrive at destination with the uninfected portion of the berry still edible. A berry infected with Botrytis or Phytophthora when shipped is usally so rotten as to be wholly inedible on arrival. The leather rot (Phytophthora rot) is the only one of the four brown rots to cause a marked vascular discoloration.

The surface of berries affected with Phytophthora under moist conditions becomes covered with a pure white growth of hyphae and conidiophores (Pl. 1, J). Those affected with Botrytis develop the gray mold which consists in part of branched conidiophores. The Pezizella rot develops little disc-shaped fruiting bodies sometimes called sporodochia. The Rhizoctonia hard rot develops only light brown, fluffy hyphae.

The mycelium of Botrytis penetrates the host cells in every direction and is both inter- and intracellular. The hyphae of the hard brown rot fungus are typically intercellular, but they are often found within the cells. Hyphae of the Pezizella are probably capable of penetrating the walls of the pulp cells but are not known to penetrate the cutinized epidermis. Little is known as to the manner in which the hyphae of the leather rot attack the host cell.

KNOWN DISTRIBUTION OF THE VARIOUS BROWN ROTS

The Rhizoctonia rot is now known from central Florida, North Carolina and possibly from Tennessee. Leather rot has been found in Mississippi, Louisiana, Arkansas, Tennessee, Missouri, Kentucky, and Illinois. Pezizella rot has been observed by the writers in Cuba, Louisiana, Florida, Arkansas, Virginia, Maryland, Wisconsin, and Alaska. Botrytis has been found to some extent as a fruit rot of strawberries in every strawberry region visited, though it varies greatly in abundance.[8] It is serious in Alaska [9] and during many seasons in New England, and may become abundant in more southern regions during wet weather. Botrytis is also known to cause rot of strawberries in England.

[8] STEVENS, NEIL E. ROTS OF EARLY STRAWBERRIES IN FLORIDA AND SOUTHERN CALIFORNIA. Amer. Jour. Bot., 9: 204-211. 1922. Literature cited, p. 211.

[9] ANDERSON, J. P., BOTRYTIS CINEREA IN ALASKA. Phytopathology. 14: 152-155. 1924.

CONTROL

Investigations as to the possibility of the control of the various field rots of strawberries by spraying and dusting are now in progress. The results which have apparently been obtained by a few growers indicate the possibility of success but it will be several years before definite recommendations can be made. Covering the ground with a mulching of pine needles is occasionally practiced in Florida, and apparently results in a lessening of the rot caused by the Rhizoctonia.

SUMMARY

A fruit rot of strawberries found to be important in central Florida is caused by a fungus which in its mycelial characters resembles *Rhizoctonia solani* Kühn as commonly found on cultivated plants.

When the berry is first attacked by this fungus the cells of the invaded tissues become somewhat hypertrophied. Later the cells of the decaying tissues gradually collapse and the mycelium of the fungus which is at first intercellular becomes both inter and intracellular.

The "hard brown rot" caused by this Rhizoctonia is characterized by the fact that it regularly starts on the under side of the berry, advances slowly and shows a definite line of demarcation between the brown decayed portion and the normal uninfected pulp. In addition the berry is often distorted or "one-sided" in shape and particles of soil usually adhere tightly to the affected area.

The "tan rot" caused by *Pezizella lythri* is confined to a somewhat cone-shaped region, the base of which appears as a tan-colored, slightly sunken spot on the side of the berry. This rotted portion may readily be separated almost intact from the sound tissue.

The leather rot, caused by a species of Phytophthora is easily distinguished, if the fruit is cut open, by the fact that the vascular tissue becomes markedly discolored being a much deeper brown than the affected pulp. Berries rotted by this fungus are very bitter to the taste.

The Botrytis or grey mold rot is not localized in any part of the berry as the fungus grows readily in all tissues. Berries affected by Botrytis are brown and rather tough without either the marked vascular discoloration or localized decay which are characteristic of one or more of the other brown rots discussed in this paper.

PLATE 1

A.—Missionary strawberry affected with the Rhizoctonia hard brown rot. The blackish discoloration of the berry is due in part to the color of the sandy soil adhering. The particles of soil are held together and bound to the berry by the mycelium of the fungus. The brown discoloration due to the rot shows more clearly next to the calyx.

B.—Cross section of the berry shown in A. Here the very dark brown band above consists of a rather hard felty mass made up of mycelium and host cells overlaid with black soil. The lighter brown layer of pulp shows to what point the fungus has penetrated.

C.—Another Missionary strawberry characteristically marked as a result of infection by the Rhizoctonia.

D.—Early stages of infection of berry with the hard brown rot. The true color of the rot is not masked by adhering soil in this case.

E.—Green berry one-third grown attacked where it lay in contact with the soil.

F.—Diseased immature berry from the Washington markets after shipment by express from Florida under refrigeration. Pure cultures of the Rhizoctonia were obtained from this berry by a transfer of diseased pulp.

G.—Section of berry two days after inoculation with the Rhizoctonia by laying a piece of agar bearing mycelium on the surface of the berry.

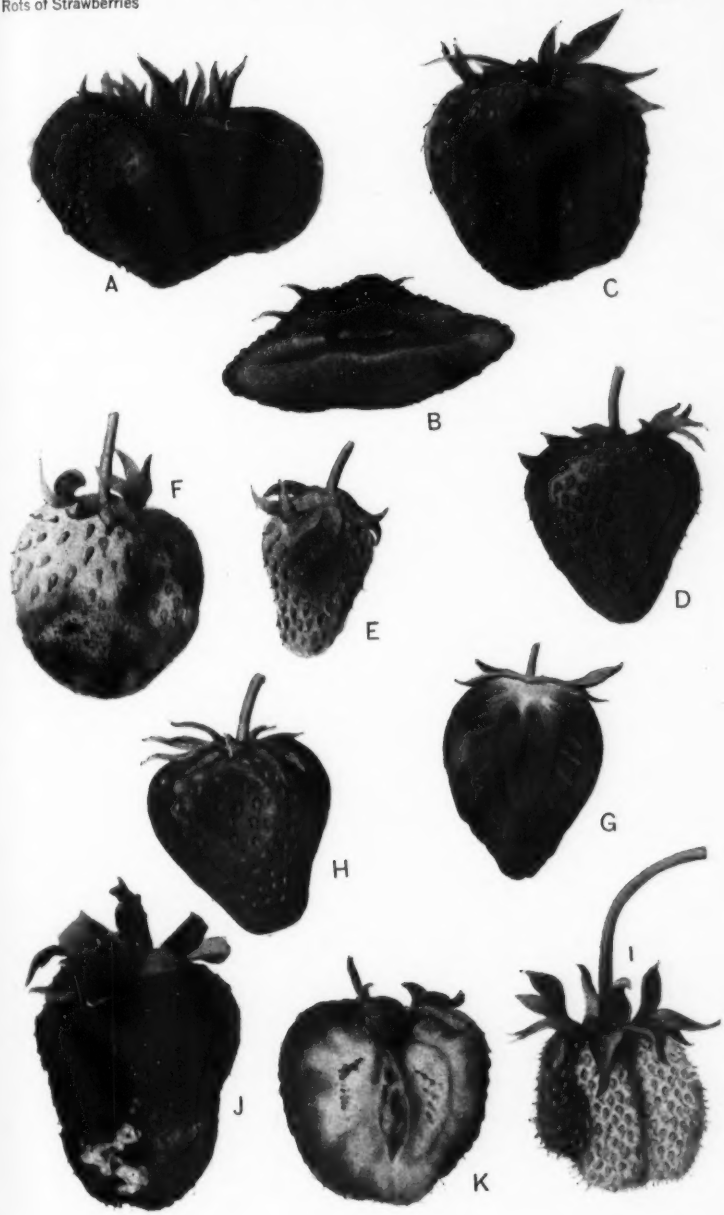
H.—Strawberry showing the tan brown rot caused by Pezizella. Note the sunken area.

I.—Green berry a short time after infection by Botrytis. Note the brown discoloration of the fruit beneath the gray mould.

J.—Aroma strawberry from Arkansas affected with the Phytophthora Leather Rot.

K.—Section of the same kind of berry showing vascular browning.

Figures A to I from colored drawings by J. Marion Shull; figures J and K, from colored photographs contributed by Dr. D. H. Rose.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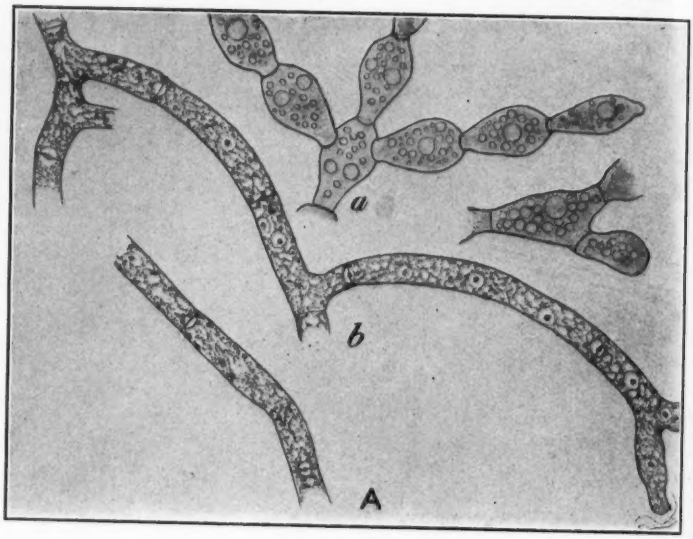
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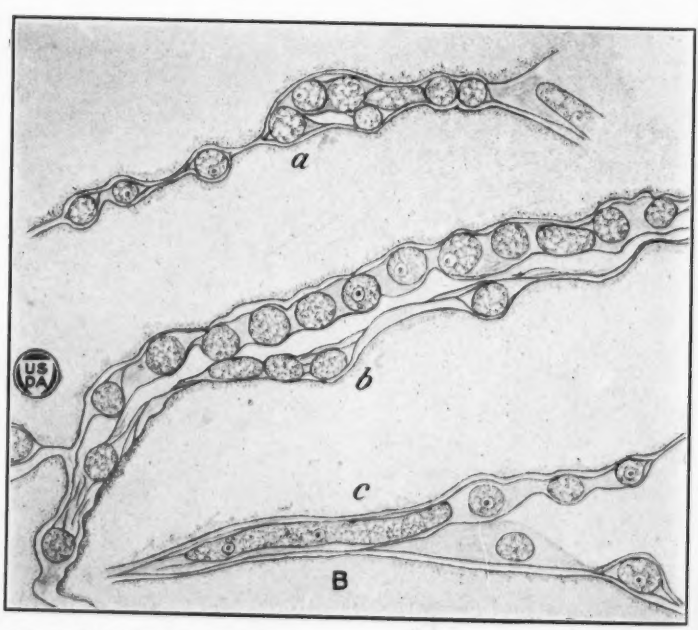
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PLATE 2

Mycelium of the Rhizoctonia from a sclerotium and from the large pulp cells of Missionary strawberry, drawn by the aid of the camera lucida A. Zeiss 4 mm. 8 oc.; B, C. 1.5 mm. Obj., 8 oc. Reduced about one-third.

A.—(*a*) Terminal branches from a sclerotium, unstained. Most of the cells in this case contain one or two large oil globules and several smaller ones. (*b*) Intracellular hyphae showing character of branching, crescent-shaped hyaline areas at the cross walls and two or three nuclei in each cell. Stained with Flemming's triple stain.

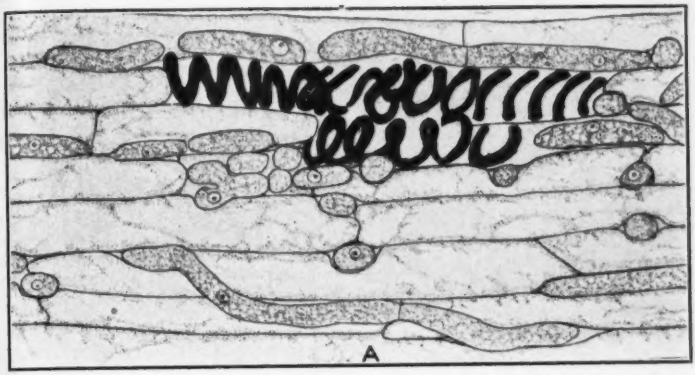
B.—(*a*) Intercellular hyphae in cross section pushing back the elastic walls of the turgid pulp cells. Note the intercellular substances, possibly derived from disorganized middle lamellae; (*b*) similar to the preceding except that the intercellular substances appear to be in more or less distinct layers. Cytoplasmic contents of pulp cells indicated by stippling. Large vacuoles filled with cell sap. Intercellular substance or disorganizing laminae of the walls shown by wash drawing; (*c*) hyphae pushing forward between two cells which at the right have spread apart to form an open intercellular space.

PLATE 3

Mycelium of the Rhizoctonia in the tissues of Missionary strawberry. Drawn with the aid of a camera lucida, Zeiss, 1.5 mm. Obj., 8 oc. Reduction about one-third.

A.—Section along the edge of a vascular bundle in the pulp. Hyphae push in between the long conducting cells forcing their walls aside. No intercellular spaces are present owing to the turgescence of the affected cells. Hyphae penetrate the vascular tissues in all directions.

B.—Section just beneath an akene, showing how the very small pulp cells in this region are entirely surrounded by intercellular hyphae which push up closely to the fruit or seed coats whose cells are thickened or sclerotized. No nuclei are visible in the host cells.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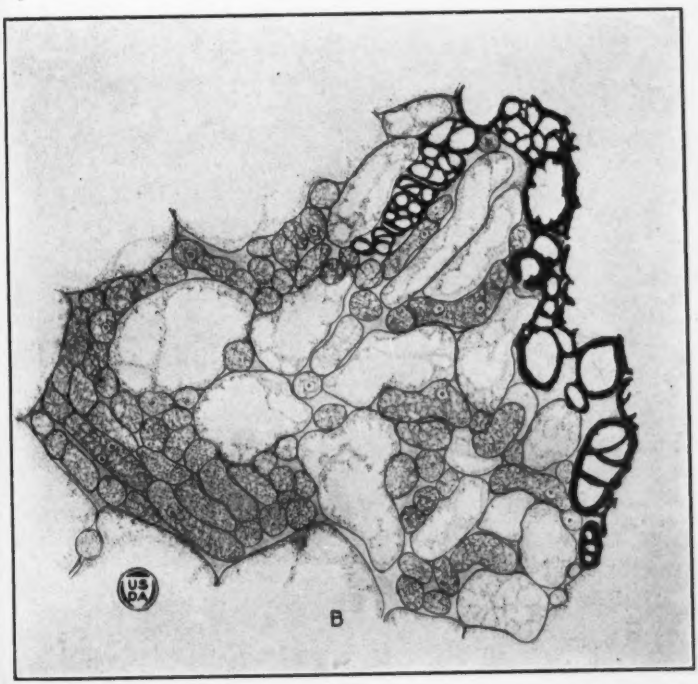
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EFFECTIVE USE OF HYDROCYANIC-ACID GAS IN THE PROTECTION OF CHICK-PEAS (CICER ARIETINUM) WAREHOUSED IN 240-POUND SACKS [1]

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One of the desiderata connected with the protection from insect attack of food commodities in storage is complete information regarding the penetration of fumigants. Hydrocyanic-acid gas is recognized as an effective and standard fumigant for the control of many pests under a wide range of conditions, but always in situations where the gas can be confined sufficiently long to reach and kill the insects. This fumigant as ordinarily used is most effective in the treatment of containers that are reasonably empty; in other words, in fumigation work which requires no great penetration of the gas. It is used with excellent results in the fumigation of houses, hospitals, railway passenger and sleeping cars, certain freight cars, ships from which cargoes have been removed, flour mills and similar structures. It is generally conceded that in the protection of commodities stored in bulk, such as wheat and corn in farmers' bins, grain cars or commercial elevators, or stored in sacks and stacked tightly in warehouses, as in the case of many animal feeds, rice, and other seeds, hydrocyanic-acid gas is far less effective and can not be depended upon for sufficient penetration to rid the commodity of its living insect inhabitants.

The use, however, of hydrocyanic-acid gas is much to be desired over that of carbon disulphid, which is a standard fumigant for such commodities, inasmuch as it can be used in large bulks in congested city districts where the generation of an equally large volume of the inflammable and explosive carbon disulphid gas would be quite out of question because of the fire hazard. For these reasons data bearing upon the power of penetration of hydrocyanic-acid gas into the usual warehoused commodities habitually affected by insects have a most practical application in conservation work. Food commodities in storage are of great value, under private ownership, subject to removal on short notice from storage and to shipment according to trade conditions, and therefore are not often available for prolonged scientific study. These are facts which enhance the rare opportunities that are offered Federal investigators to study privately owned stored products.

An unusual opportunity was presented the writers during the years 1918–1920 to observe the disastrous attack of the four-spotted bean weevil *Bruchus quadrimaculatus*, the lesser grain borer *Rhizopertha dominica*, the rice weevil *Sitophilus oryza*, the Angoumois grain moth *Sitotroga cerealella*, the Indian meal moth *Plodia interpunctella*, and the tobacco beetle *Lasioderma serricorne*, upon a large supply of chick-peas, *Cicer arietinum*. These edible legumes, amounting to about 137,000 sacks of 240 pounds each, represented for the most part the greater portion of the crop of this food grown in northern Mexico during 1918 and diverted from storage in New York City by congested war conditions to six warehouses in New Orleans. This crop had arrived at New Orleans by rail during the summer of 1918 and had been stored under both favorable and unfavorable conditions in space permitted in lots of 15,000, 12,000, 30,000, 30,000, 30,000, and 20,000 sacks, respectively, in the six warehouses. During

[1] Received for publication April 22, 1924.

early October, 1918, the owners discovered that while the bulk of the consignments was uninfested, many carload lots of the seeds showed heavy infestation and were heating badly as a result of bruchid attack. The centers of infestation were, unfortunately, so distributed throughout the warehouses that rapid spread in infestation was taking place, and the heating caused by the infestations was offsetting the normal checks of colder winter weather upon weevil activity and spread. Numerous sacks of seeds had already been rendered unfit for food both by the bruchids and the Rhizopertha. Since the seeds had a retail value of about $5,000,000, action was imperative. As the warehouses, with one exception, were within the congested business districts of the city only a noninflammable gas could be used. Hydrocyanic-acid gas was chosen by the owners, who engaged a professional fumigator. This operator fumigated the warehouses, using 2½ pounds of sodium cyanid 98 to 99 per cent pure for each 1,000 cubic feet of space. The senior writer was present at the time of the fumigations and took numerous samples, the results of the examinations of which are only in part reported upon in this paper. The fumigations were allowed to extend over a period of 48 hours, although the warehouses were not as a rule tight enough to confine the gas that long. The writers were not permitted to experiment with the dosage chosen by the owners. While the cost of using so large a dosage greatly increased the cost of fumigation above what was necessary in the opinion of the writers, the cost was nothing in the eyes of the owners when compared to the value of the commodity. The fumigations were conducted when the average mean temperatures ranged from 58° to 75° F.

In taking samples both before and after fumigations, the writers used the usual grain car probe, about 5 feet long, as illustrated (Pl. 1, C). This probe was thrust from end to end of the sack through the central axis and the samples (usually six) placed separately in sacks and numbered according to their relative positions in the bulk of the sack sampled. In making the intensive examination later only 50 of the seeds of a sample were used, the remainder being held for future observation.

Diagrams are presented hereafter showing the location of the sacks from which samples were taken after fumigation, together with data on the degree of infestation and penetration of the gas and the effect of the fumigant upon the insects. It happens that the data on infestations serve to indicate also both the penetration and the effectiveness of the fumigant, for the reason that in no sample recorded in the following pages were any insects found alive, and no development occurred subsequently from eggs on the seeds or from larvæ or pupæ within the seeds at the time of fumigation, as shown by examination made at intervals during two years following the fumigations. No extended examinations were made until about one month after the fumigations, when the dead larvæ and pupæ had had time to discolor if killed. All seeds examined for larvæ and pupæ were first softened and then sliced with scalpels to reveal infestation. The references to the degree of larval development, such as one-eighth, one-fourth, one-half, or full-grown, are of course approximate statements based upon the general appearance of the individual.

WAREHOUSE NO. 1

The 30,000 sacks of chick-peas stored in this warehouse were in a room on the third (top) floor, measuring 150 by 150 by 20 feet. The warehouse was of brick construction, with a tar and pebble roof. Leakage of gas about the roof during fumigation was rather great, little or no discomfort being experienced when the room was opened for ventilation 48 hours after fumigation was started. The fumigator used 80 fifty-gallon oil barrels, each generating a charge containing 15 pounds of sodium cyanid. The capacity of this warehouse was greatly overtaxed (Pl. 1, A), the sacks being piled from 6 to 17 deep, whereas the usual

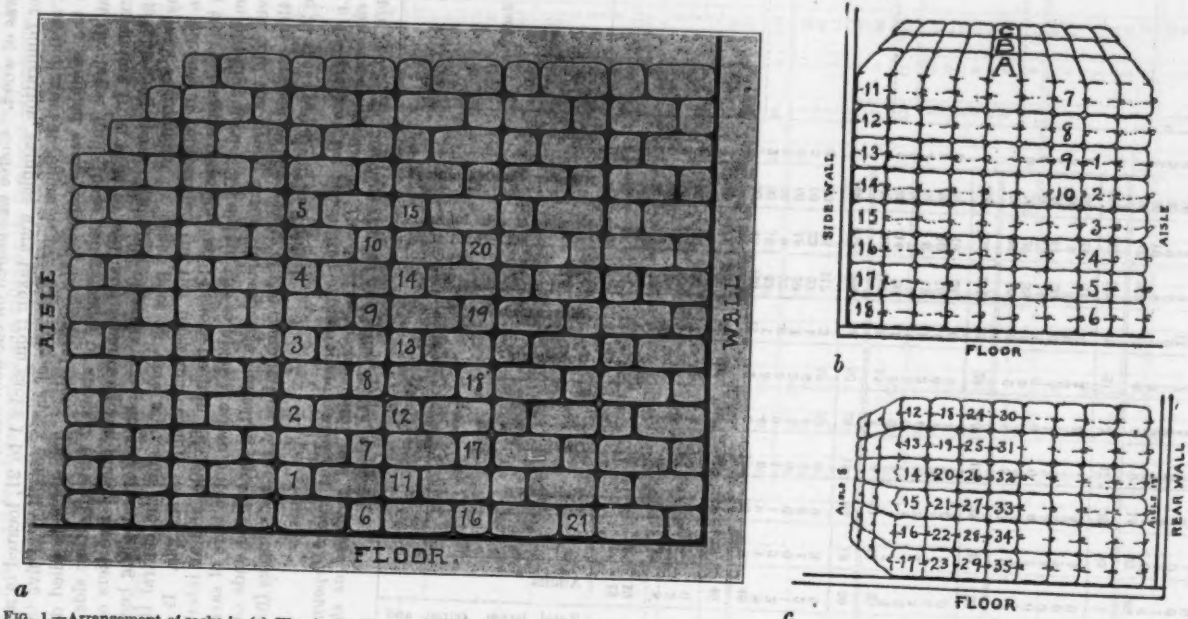


FIG. 1.—Arrangement of sacks in (a) Warehouse No. 1, (b) Warehouse No. 2, and (c) Warehouse No. 3, from which samples were taken and reported on in Tables I to III, respectively.

custom for storing this commodity is to pile the sacks but 5 or 6 deep. The floor was of wood. Coffee was stored on the floor below.

After fumigation, samples were taken from sacks 1 to 21, located in a pile of sacks, as indicated in figure 1, *a*. This pile of sacks consisted of five tiers, of which the sampled sacks formed the central tier. The lot was piled directly against the brick wall, there being spaces of about 2 feet on either side of the pile, extending from aisle to wall and separating the pile of five tiers of sacks from neighboring tiers. This pile of sacks was chosen for sampling because it was as well surrounded by other piles as were any; and the central tier was chosen because it represented the tier most inaccessible to the gas. It will be noted that the sacks were stacked 14 sacks deep in this pile, and an interlocking system of piling used. To obtain the samples the two outer tiers of sacks were removed and the probe thrust through the indicated sacks, the ends of which were thus exposed. The results of the examination of the six samples from each of the 21 sacks are given in Table I.

TABLE I.—*Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by Bruchus quadrimaculatus and arranged as shown in figure 1, a. All bruchids reached and killed by the fumigant*

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—									
						Larvæ							
				Eggs	Emergence holes	One-eighth	One-fourth	One-half	Full-grown	Pupæ	Adults	Total larvæ, pupæ, and adults	Eggs unaccounted for
1	1	50	47	321	14	39	19	17	70	17	19	181	126
	2	50	45	167	1	31	8	16	30	3	10	98	158
	3	50	12	43	0								43
	4	50	26	19	3	1	1	0	5	3	8	18	
	5	50	26	57	3	13	2	4	4	2	3	28	26
	6	50	21	150	2	13	3	4	5	2	0	27	121
		300	177	757	23	97	33	41	114	27	40	352	473
2	1	50	48	140	19	2	6	10	36	5	46	105	16
	2	50	18	43	1	5	4	6	9	1	8	33	9
	3	50	9	22	6	3	0	1	1	2	2	9	7
	4	50	15	20	3	3	3	0	0	0	1	7	10
	5	50	22	27	1	5	4	1	8	1	2	21	5
	6	50	40	117	3	42	22	0	9	3	0	76	38
		300	152	369	33	60	39	18	63	12	59	251	85
3	1	50	43	185	18	13	5	8	21	15	25	87	80
	2	50	14	20	1	1	0	1	1	3	2	8	12
	3	50	7	13	1	1	0	1	1	1	3	7	5
	4	50	8	12	1	2	0	1	2	1	3	9	2
	5	50	10	15	4	0	1	2	3	1	5	12	
	6	50	19	26	0	6	4	3	0	3	0	16	10
		300	101	271	25	23	10	16	28	24	38	139	109
4	1	50	38	60	3	2	8	8	15	6	0	39	13
	2	50	26	38	5	3	0	5	3	7	1	19	14
	3	50	12	14	1	6	0	0	0	2	0	8	5
	4	50	14	20	0	4	1	2	2	3	0	12	8
	5	50	8	8	0	3	1	1	3	2	0	10	
	6	50	14	17	0	3	5	1	3	5	0	17	0
		300	112	157	9	21	15	17	26	25	1	105	45
5	1	50	47	238	42	6	5	9	14	27	69	130	66
	2	50	10	9	5	3	0	0	1	0	4	8	
	3	50	9	9	0	1	0	2	0	1	1	5	4
	4	50	7	4	11	2	1	2	4	0	0	9	
	5	50	10	5	10	2	2	3	2	1	0	10	
	6	50	12	6	20	3	2	3	6	1	2	17	
		300	95	271	88	17	10	19	27	30	76	179	70

TABLE I.—*Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by Bruchus quadrimaculatus and arranged as shown in figure 1, a. All bruchids reached and killed by the fumigant*—Continued

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—									
				Eggs	Emergence holes	Larvæ				Pupæ	Adults	Total larvæ, pupæ, and adults	Eggs unaccounted for
						One-eighth	One-fourth	One-half	Full-grown				
6	1	50	30	91	1	12	11	7	6	6	4	46	44
	2	50	22	42	2	10	2	8	1	3	3	27	13
	3	50	19	22	3	3	0	6	4	1	0	14	5
	4	50	11	13	0	3	2	0	2	0	0	7	6
	5	50	28	56	0	1	0	3	0	0	0	4	52
	6	50	38	134	0	13	9	1	4	1	0	28	106
		300	148	358	6	42	24	25	17	11	7	126	226
7	1	50	46	230	32	7	4	2	13	12	18	56	142
	2	50	34	71	9	5	3	3	6	8	5	30	32
	3	50	16	19	0	1	1	1	4	3	0	10	9
	4	50	24	37	0	12	4	1	5	2	1	25	12
	5	50	31	49	0	27	1	2	6	2	1	39	10
	6	50	29	55	1	18	0	1	6	0	0	25	29
		300	180	461	42	70	13	10	40	27	25	185	234
8	1	50	41	173	12	10	8	17	33	24	9	101	60
	2	50	22	31	3	3	2	3	5	6	3	22	6
	3	50	8	10	0	3	2	0	2	1	0	8	2
	4	50	2	2	0	1	0	0	1	0	0	2	0
	5	50	2	2	0	0	0	0	0	1	0	1	1
	6	50	3	3	0	2	0	0	0	1	0	3	0
		300	78	221	15	19	12	20	41	33	12	137	69
9	1	50	0	0	0	0	0	0	0	0	0	0	0
	2	50	0	0	0	0	0	0	0	0	0	0	0
	3	50	1	1	0	0	0	0	0	0	0	0	1
	4	50	7	7	1	0	0	0	0	0	0	0	6
	5	50	16	32	0	8	9	6	3	1	0	27	5
	6	50	44	108	0	22	16	11	18	15	5	87	21
		300	68	148	1	30	25	17	21	16	5	114	33
10	1	50	44	180	3	19	4	7	17	7	4	58	119
	2	50	33	83	0	19	4	13	4	5	2	47	36
	3	50	16	24	1	5	3	1	2	0	2	13	10
	4	50	17	19	0	4	0	1	0	2	1	8	11
	5	50	25	37	0	5	10	1	3	2	2	23	14
	6	50	35	63	0	14	4	6	2	7	0	33	30
		300	170	406	4	66	25	29	28	23	11	182	220
11	1	50	35	77	0	30	0	7	4	1	0	42	35
	2	50	13	26	0	7	0	0	3	3	0	13	13
	3	50	18	27	0	13	0	3	0	3	0	19	8
	4	50	37	65	0	14	2	10	4	3	0	33	32
	5	50	38	75	1	29	0	14	6	4	1	54	20
	6	50	36	64	2	32	3	5	3	3	4	50	12
		300	177	334	3	125	5	39	20	17	5	211	120
12	1	50	41	114	21	5	2	1	7	17	16	48	45
	2	50	25	35	5	5	1	0	1	2	6	15	15
	3	50	7	7	1	0	0	0	3	1	3	7	------
	4	50	19	30	1	1	2	2	5	3	1	14	15
	5	50	19	24	0	8	3	1	0	7	2	21	3
	6	50	12	14	0	1	3	2	2	2	0	10	4
		300	123	224	28	20	11	6	18	32	28	115	82
13	1	50	29	74	2	11	7	7	12	10	2	49	25
	2	50	2	8	5	2	0	0	1	0	3	6	------
	3	50	3	3	0	1	0	1	2	0	0	4	------
	4	50	4	4	3	0	1	0	1	0	0	2	------
	5	50	3	9	4	0	0	2	4	1	0	7	------
	6	50	11	17	1	7	2	1	1	0	1	12	4
		300	52	115	15	21	10	11	21	11	6	80	27

TABLE I.—*Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by Bruchus quadrimaculatus and arranged as shown in figure 1, a. All bruchids reached and killed by the fumigant*—Continued

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—									
				Eggs	Emergence holes	Larvæ				Pupæ	Adults	Total larvæ, pupæ, and adults	Eggs unaccounted for
						One-eighth	One-fourth	One-half	Full-grown				
14	1	50	27	67	12	15	13	0	0	0	14	42	13
	2	50	12	14	0	2	0	0	0	0	0	2	12
	3	50	17	18	2	5	1	0	2	0	1	9	7
	4	50	17	24	1	3	1	2	4	2	1	13	10
	5	50	33	54	5	2	12	6	1	7	1	29	20
	6	50	31	48	2	2	11	9	4	3	1	30	16
		300	137	225	22	29	38	17	11	12	18	125	78
15	1	50	0	0	0	0	0	0	0	0	0	0	0
	2	50	0	0	0	0	0	0	0	0	0	0	0
	3	50	1	1	0	1	0	0	0	0	0	1	0
	4	50	2	2	0	1	0	0	0	0	0	1	1
	5	50	3	3	0	0	0	0	2	0	0	2	1
	6	50	11	12	0	3	1	1	2	1	0	8	4
		300	17	18	0	5	1	1	4	1	0	12	6
16	1	50	50	321	8	22	14	13	34	31	32	146	167
	2	50	28	59	3	2	0	3	4	12	3	24	33
	3	50	19	19	0	4	0	0	0	1	1	6	13
	4	50	41	80	6	9	1	16	9	9	7	51	23
	5	50	39	58	0	21	4	7	4	1	1	38	20
	6	50	48	138	0	57	18	18	5	2	1	101	37
		300	225	675	17	115	37	57	56	56	45	366	292
17	1	50	40	103	8	5	9	7	8	11	12	52	43
	2	50	40	80	2	22	0	0	4	1	0	27	51
	3	50	34	53	1	5	0	0	0	1	0	6	46
	4	50	30	44	1	15	0	2	1	0	1	19	24
	5	50	34	47	4	17	8	1	1	0	1	28	15
	6	50	41	91	0	13	4	1	6	6	0	30	61
		300	219	418	16	77	21	11	20	19	14	162	240
18	1	50	14	18	0	4	1	5	0	0	0	10	8
	2	50	19	23	1	1	1	2	1	0	0	5	17
	3	50	9	10	0	0	1	1	2	4	0	8	2
	4	50	11	11	0	2	2	5	1	1	0	11	0
	5	50	7	8	0	1	2	1	1	2	0	7	1
	6	50	6	7	0	1	0	1	2	1	0	5	2
		300	66	77	1	9	7	15	7	8	0	46	30
19	1	50	44	177	18	24	22	12	39	37	21	155	4
	2	50	19	33	5	2	6	1	6	3	3	21	7
	3	50	15	21	4	3	1	3	3	1	2	13	4
	4	50	3	3	1	0	0	1	0	1	0	2	0
	5	50	3	7	5	1	0	3	0	1	0	5	----
	6	50	8	9	1	3	0	1	3	0	1	8	0
		300	92	250	34	33	29	21	51	43	27	204	15
20	1	50	4	4	0	1	1	1	0	1	0	4	0
	2	50	0	0	0	0	0	0	0	0	0	0	0
	3	50	3	3	0	1	1	0	1	0	0	3	0
	4	50	5	5	1	1	0	0	1	0	1	3	1
	5	50	13	17	1	3	3	0	4	0	1	11	5
	6	50	13	15	0	4	5	0	3	1	0	13	2
		300	38	44	2	10	10	1	9	2	2	34	8
21	1	50	49	226	8	36	35	18	37	11	1	138	80
	2	50	45	131	0	45	29	6	14	1	0	95	36
	3	50	35	60	0	38	2	1	3	0	0	44	16
	4	50	24	29	0	24	0	0	0	0	0	24	5
	5	50	28	35	0	14	0	0	1	0	0	15	20
	6	50	13	15	0	3	0	0	2	0	0	5	10
		300	194	496	8	160	66	25	57	12	1	321	167

WAREHOUSE No. 2

The 30,000 sacks of chick-peas stored in this two-story brick warehouse were stacked on both floors, each floor measuring 150 by 50 by 10 feet, with wooden floors, and with large sliding doors on the ground floor opening on the street on either side of the warehouse. Both floors were fumigated as a single unit. The sacks were stacked from 8 to 10 deep. The sacks sampled were on the ground floor and located as indicated in figure 1, *b*, being well toward the rear of the warehouse between a side aisle and a brick wall, the tier A being 15 rows or tiers from the street wall. Sacks 1 to 6 were in tier B; sacks 7 to 10 in tier C; sacks 11 to 18 in tier A. Tiers of sacks on either side of a block A–B–C, and a portion of the sacks of tier A were removed to make possible the taking of samples. While this warehouse was well constructed, considerable leakage of gas took place through the paper pasted over the iron gratings above the doors. It is believed by the writers that sacks 11 to 18 stacked against the rear brick wall were located in as difficult a position as any from the standpoint of gas penetration. The results of the examination of the samples taken in warehouse No. 2 are recorded in Table II.

TABLE II.—*Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by Bruchus quadrimaculatus and arranged as shown in figure 1, b. All bruchids reached and killed by the fumigant*

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—									
				Eggs	Emergence holes	Larvæ				Pupæ	Adults	Total larvæ, pupæ, and adults	Eggs unaccounted for
						One-eighth	One-fourth	One-half	Full-grown				
1	1	50	49	308	38	3	5	14	9	16	17	64	206
	2	50	42	191	3	4	7	5	9	31	28	84	104
	3	50	27	67	5	13	1	5	9	8	2	38	24
	4	50	20	26	0	1	1	1	1	0	0	4	22
	5	50	9	11	0	0	0	0	0	0	0	0	11
	6	50	4	6	0	2	0	0	0	0	0	2	4
		300	151	609	46	23	14	25	28	55	47	192	371
2	1	50	48	243	2	22	12	5	5	3	10	57	184
	2	50	46	353	7	2	0	4	17	4	28	55	291
	3	50	48	328	4	31	0	16	13	21	31	112	212
	4	50	49	327	4	7	3	16	7	27	15	75	248
	5	50	49	444	3	173	66	20	25	17	5	306	133
	6	50	23	44	0	5	1	5	9	3	5	28	16
		300	263	1,739	22	240	82	66	76	75	94	633	1,084
3	1	50	50	282	16	38	3	15	19	29	2	106	160
	2	50	41	104	3								101
	3	50	16	28	0	9	3	3	1	14	0	30	
	4	50	5	5	0	2	2	1	0	0	0	5	0
	5	50	2	2	0	0	2	0	0	0	0	2	0
	6	50	10	13	0	4	0	2	1	1	0	8	5
		300	124	434	19	53	10	21	21	44	2	151	266
4	1	50	42	124	2	17	3	6	30	10	6	72	50
	2	50	40	104	4	13	11	10	24	2	6	66	34
	3	50	36	76	2								74
	4	50	39	82	1	19	2	4	1	2	6	34	47
	5	50	30	49	4	16	1	0	2	2	2	23	22
	6	50	25	38	1	10	1	1	0	1	1	14	23
		300	212	473	14	75	18	21	57	17	21	209	250
5	1	50	6	5	0	1	0	0	1	1	1	4	1
	2	50	1	3	0	0	0	0	1	1	0	2	1
	3	50	2	3	0	0	0	0	0	0	0	0	3
	4	50	3	3	0	0	0	0	0	1	0	1	2
	5	50	25	49	0	4	2	4	5	2	1	18	31
	6	50	37	89	4	24	6	1	5	11	4	51	34
		300	74	152	4	29	8	5	12	16	6	76	72

TABLE II.—*Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by Bruchus quadrimaculatus and arranged as shown in figure 1, b. All bruchids reached and killed by the fumigant*—Continued

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—								
				Eggs	Emergence holes	Larvæ				Pupæ	Adults	Total larvæ, pupæ, and adults	Eggs unaccounted for
						One-eighth	One-fourth	One-half	Full-grown				
6	1	50	24	30	1	3	1	0	6	6	3	19	10
	2	50	9	9	0	0	0	0	0	0	0	0	9
	3	50	0	0	0	0	0	0	0	0	0	0	0
	4	50	0	0	0	0*	0	0	0	0	0	0	0
	5	50	2	2	0	0	0	0	0	0	0	0	2
	6	50	10	11	0	4	1	0	1	0	0	6	5
		300	45	52	1	7	2	0	7	6	3	25	26
7	1	50	43	226	6	20	5	19	46	38	24	152	68
	2	50	29	62	4	4	0	8	24	12	3	51	7
	3	50	22	43	0	26	3	0	1	0	0	30	13
	4	50	38	70	2	51	4	5	2	0	0	62	6
	5	50	47	232	1	72	1	0	1	0	1	75	156
	6	50	48	343	5	59	7	34	60	20	13	193	145
		300	227	976	18	232	20	66	134	70	41	563	395
8	1	50	49	290	6	17	10	10	9	2	1	49	235
	2												
	3	50	30	66	0	13	4	2	4	1	0	24	42
	4	50	19	25	1	3	1	1	1	2	2	10	14
	5	50	23	31	2	9	0	2	2	1	1	15	14
	6												
		200	121	412	9	42	15	15	16	6	4	98	305
9	1	50	45	206	3	11	20	19	17	28	16	111	92
	2	50	50	220	8	59	29	13	12	17	9	139	73
	3	50	14	16	3	2	0	1	0	0	2	5	8
	4	50	5	7	0	0	2	0	0	0	1	3	4
	5	50	9	10	0	0	0	0	0	0	0	0	10
	6	50	27	54	0	9	5	0	5	0	0	19	35
		300	150	513	14	81	56	33	34	45	28	277	223
10	1	50	48	353	44	30	9	2	8	84	23	156	153
	2	50	32	98	14	13	5	7	5	31	4	65	19
	3	50	11	15	1	5	4	0	1	5	1	16	
	4	50	5	5	1	0	0	2	5	5	4	16	
	5												
	6	50	37	106	2	19	6	8	5	5	2	45	59
		250	133	577	62	67	24	19	24	130	34	298	231
11	1	50	50	257	15	21	6	31	55	30	25	168	74
	2	50	50	187	6	59	17	10	12	3	3	104	77
	3	50	49	129	2	71	5	5	5	1	3	90	37
	4	50	28	41	0								41
	5	50	27	46	0	27	0	0	0	0	0	27	19
	6	50	40	71	0	36	3	0	0	0	0	39	32
		300	244	731	23	214	31	46	72	34	31	428	280
12	1	50	22	30	0	16	10	1	0	0	0	27	3
	2	50	14	18	0	0	0	0	1	2	0	3	15
	3	50	4	5	0	1	1	1	0	0	0	3	2
	4	50	2	2	0	0	2	0	0	1	0	3	
	5	50	2	2	0								2
	6	50	14	15	0	8	0	1	0	0	0	9	6
		300	58	72	0	25	13	3	1	3	0	45	28
13	1	50	49	329	18	51	32	18	7	2	4	114	197
	2	50	42	156	6	61	9	3	0	1	1	75	76
	3	50	22	34	1	22	0	0	1	0	1	24	9
	4	50	9	10	0	6	1	0	0	1	1	9	1
	5	50	13	16	0	4	2	0	0	0	0	6	10
	6	50	20	29	0								29
		300	155	574	24	144	44	21	8	4	7	228	323

TABLE II.—*Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by Bruchus quadrimaculatus and arranged as shown in figure 1, b. All bruchids reached and killed by the fumigant*—Continued

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—									
				Eggs	Emergency holes	Larvæ				Pupæ	Adults	Total larvæ, pupæ, and adults	Eggs unaccounted for
						One-eighth	One-fourth	One-half	Full-grown				
14	1	50	50	333	36	18	62	36	34	11	19	180	117
	2	50	47	211	1	80	9	1	1	0	0	91	119
	3	50	24	43	0	10	4	1	0	0	0	15	28
	4	50	21	27	0	8	0	0	0	0	0	8	19
	5	50	5	6	0	0	0	0	0	0	0	0	6
	6	50	3	7	1	0	0	2	0	0	2	4	2
		300	150	627	38	116	75	40	35	11	21	298	291
15	1	50	50	588	2	137	11	13	6	6	9	182	404
	2	50	47	373	2	82	6	7	3	1	12	111	260
	3	50	50	429	2	63	3	4	4	1	5	80	347
	4	50	49	343	3	51	0	0	1	0	3	55	285
	5	50	48	399	1	88	10	5	0	2	5	110	288
	6	50	49	639	------	12	15	32	29	40	148	276	363
		300	293	2,771	10	433	45	61	43	50	182	814	1,947
16	1	50	49	311	10	36	28	23	10	25	9	131	170
	2	50	40	90	0	2	0	0	2	0	0	4	86
	3	50	10	16	0	0	0	0	0	0	0	0	16
	4	50	9	14	0	3	1	0	0	0	0	4	10
	5	50	14	26	0	1	2	2	1	1	1	8	18
	6	50	46	171	1	21	11	1	7	2	3	45	125
		300	168	628	11	63	42	26	20	28	13	192	425
17	1	50	46	136	21	1	4	22	58	50	32	167	------
	2	50	20	54	1	------	------	------	------	------	------	------	54
	3	50	18	24	2	2	0	1	4	4	3	15	7
	4	50	16	25	0	4	3	0	2	2	1	11	14
	5	50	14	16	0	------	------	------	------	------	------	------	16
	6	50	25	42	3	11	5	0	1	1	0	18	21
		300	139	297	27	18	12	23	65	57	36	211	112
18	1	50	46	156	16	3	3	8	42	33	22	111	29
	2	50	30	55	0	14	4	1	7	5	2	33	22
	3	50	12	16	0	0	0	1	3	0	0	4	12
	4	50	8	10	0	4	0	0	0	0	0	4	6
	5	50	14	18	0	3	0	0	0	0	0	3	15
	6	50	17	25	0	4	0	0	0	0	0	4	21
		300	127	280	16	28	7	10	52	38	24	159	105

WAREHOUSE No. 3

The 30,000 sacks of chick peas stored in this warehouse were stacked as indicated in Plate 1, B. The warehouse had a wooden frame covered with sheets of galvanized iron. The cracks of the building were papered over by professional paper hangers with bill posters. This warehouse was extremely loose in construction, with so many openings that the writers would not have believed that it could be made sufficiently tight even with the aid of professional paperers.

Badly infested sacks were found heating on both floors of this warehouse. While the samples recorded were taken from the second floor, infestations at *a* of Plate 1, B, were found equally well killed by the fumigation. The sacks shown in figure 1, *c*, represent one carload of chick-peas. Sacks No. 12 to 35 were exposed at the ends and numbered, on a 13-inch side aisle, between stacks of carload lots, leading from the main 8-foot aisle to the rear wall.

The data of Table III record the number of bruchid eggs and emergence holes found in the samples taken from sacks 12 to 35, and thus, by comparison with

the detailed data of Tables I and II, indicate that the 50 seeds of each sample were in some instances heavily infested. Upon examination all stages of the bruchids were found dead in seeds, and no development occurred in seeds held for subsequent observation.

TABLE III.—*Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by Bruchus quadrimaculatus and arranged as shown in figure 1, c. All bruchids reached and killed by the gas*

Sack No.	Sample No.	Number of—		Sack No.	Sample No.	Number of—		Sack No.	Sample No.	Number of—	
		Eggs	Emergence holes			Eggs	Emergence holes			Eggs	Emergence holes
12	1	98	4	13	1	97	2	14	1	222	10
	2	94	2		2	52	0		2	155	6
	3	67	2		3				3	118	8
	4	29	0		4	44	1		4	93	6
	5	93	3		5	79	3		5	95	11
	6	164	6		6	101	7		6	111	13
15	1	260	14	16	1	94	10	17	1	429	17
	2	94	15		2	72	8		2	228	2
	3	90	4		3	97	7		3	213	3
	4	107	5		4	135	15		4	247	8
	5	110	7		5	159	13		5	291	7
	6	152	0		6	291	39		6	383	12
18	1	45	0	19	1	358	43	20	1	528	33
	2	41	1		2	114	5		2	196	2
	3	48	4		3	86	1		3	75	2
	4	73	4		4	47	0		4	42	1
	5	69	1		5	45	0		5	32	1
	6	75	3		6	56	0		6	82	5
21	1	54	10	22	1	389	33	23	1	108	2
	2	45	7		2	204	3		2	64	1
	3	42	4		3	45	2		3	98	3
	4	32	3		4	40	6		4	199	2
	5				5	56	3		5	331	3
	6	205	50		6	39	6		6	498	19
24	1	315	18	25	1	321	7	26	1	291	4
	2	137	15		2	214	16		2	307	10
	3	58	2		3	128	2		3	146	6
	4	55	2		4	33	2		4	89	2
	5	34	4		5	37	0		5	69	1
	6	47	0		6	41	1		6	47	4
27	1	115	12	28	1	313	13	29	1	132	8
	2	98	7		2	300	3		2	84	3
	3	83	2		3	60	2		3	69	2
	4	131	2		4	39	1		4	64	1
	5	277	13		5	72	0		5	157	2
	6	195	6		6	73	0		6	334	27
30	1	128	3	31	1	126	1	32	1	292	26
	2	60	2		2	52	1		2		
	3	67	1		3	52	0		3		
	4	104	1		4	30	0		4		
	5	165	4		5	41	0		5		
	6	227	6		6	57	3		6		
33	1			34	1	83	1	35	1	201	11
	2	81	2		2	53	0		2	120	3
	3	35	0		3	51	0		3	119	0
	4	37	1		4	78	1		4	76	4
	5	31	0		5	178	7		5	73	5
	6	49	0		6	206	2		6	132	9

INSECTS OTHER THAN BRUCHIDS

The data on infestations recorded above deal only with *Bruchus quadrimaculatus*. In Warehouse No. 4, of brick construction and very tight, about 110 by 66 by 13 feet, the sacks were stacked only four and five deep. The seeds in them contained some infestations by bruchids, but were more interesting because of severe infestations by *Rhizopertha dominica* and much slighter infestations by *Lasioderma sericorne*. The Rhizopertha infestations had in certain sacks reduced the seeds to a condition rendering them easily crushed between the fingers. The Lasioderma infestations were primary but with never more than one or two larvæ or pupæ in a seed. This warehouse was fumigated in the same manner as Warehouses 1 to 3, with the result that examinations of

the seeds made with the same tedious carefulness revealed no living specimens of larvæ, pupæ, or adults.

In all five warehouses fumigated there were found slight infestations of *Sitophilus oryza*, *Plodia interpunctella*, and *Sitotroga cerealella*. The Plodia infestations appeared to be easily killed by the fumigations. Large numbers of the Sitophilus and Sitotroga were killed, but so many were not that the fumigations, from the standpoint of their control, could not be called a success. It should be said, in passing, that Sitophilus and Sitotroga infestations are not usual in chick-pea stocks, bruchids being the only really serious pest, aside from the infrequent and devastating attacks of the Rhizopertha.

OBSERVATIONS MADE SUBSEQUENT TO FUMIGATION

Many samples of seeds were taken from all warehouses just previous to fumigation (November 29, 1918). These were examined seven months later (June, 1919). All contained many living adult and immature bruchids and their parasites, except a few of the more heavily infested samples which had become too badly damaged to support a bruchid infestation, with the result that the infestations had burned themselves out.

Of the hundreds of fumigated samples held for observation only one developed living bruchid material. This one sample showed a few living specimens on June 23, 1919. Many of the fumigated samples were held for observation in the laboratory but developed no infestations up to 1923, although conditions were favorable to bruchid development, as indicated by the growth of bruchid cultures in other containers and by the ease with which the fumigated seeds became infested in 1923 when adult bruchids were given access to them.

SUMMARY

It has been generally conceded that commodities such as wheat, corn, rice, animal feeds, flour, etc., stored in bulk in farmers' bins, grain elevators, or stacked closely in sacks in warehouses, are not satisfactorily protected by ordinary fumigation with hydrocyanic-acid gas because this gas lacks sufficient power of penetration to reach and kill insects hidden within the commodity. Since such food supplies are subject to severe insect attack in storage, usually in congested districts where inflammable and explosive gases can not be used without danger, all data bearing upon the penetration of hydrocyanic-acid gas are of value. Because food commodities in storage under private ownership are of great value, subject to removal on short notice according to trade conditions, and therefore not available for prolonged study, opportunities for studies upon the penetration and effectiveness of insecticide gases are rare and, when offered, should be improved. The data recorded on the penetration and effectiveness of hydrocyanic-acid gas during the fumigation of 137,000 sacks of chick-peas (*Cicer arietinum*) each weighing 240 pounds, the lot having a retail value of $5,000,000, are new and valuable.

No similar large-scale practical work with a warehoused commodity has previously been recorded. It was found that infestations by *Bruchus quadrimaculatus*, *Rhizopertha dominica*, *Lasioderma serricorne*, and *Plodia interpunctella* could be brought under very satisfactory control by fumigation with hydrocyanic-acid gas, as a result of the thorough penetration of the gas throughout the bulk of the 240-pound sacks, no matter whether these were stacked four or five deep, as chick-peas are ordinarily warehoused, or in piles, the tiers of which were often 18 sacks high. Infestations of *Sitophilus oryza* and *Sitotroga cerealella* were not satisfactorily controlled by one fumigation, though many were killed.

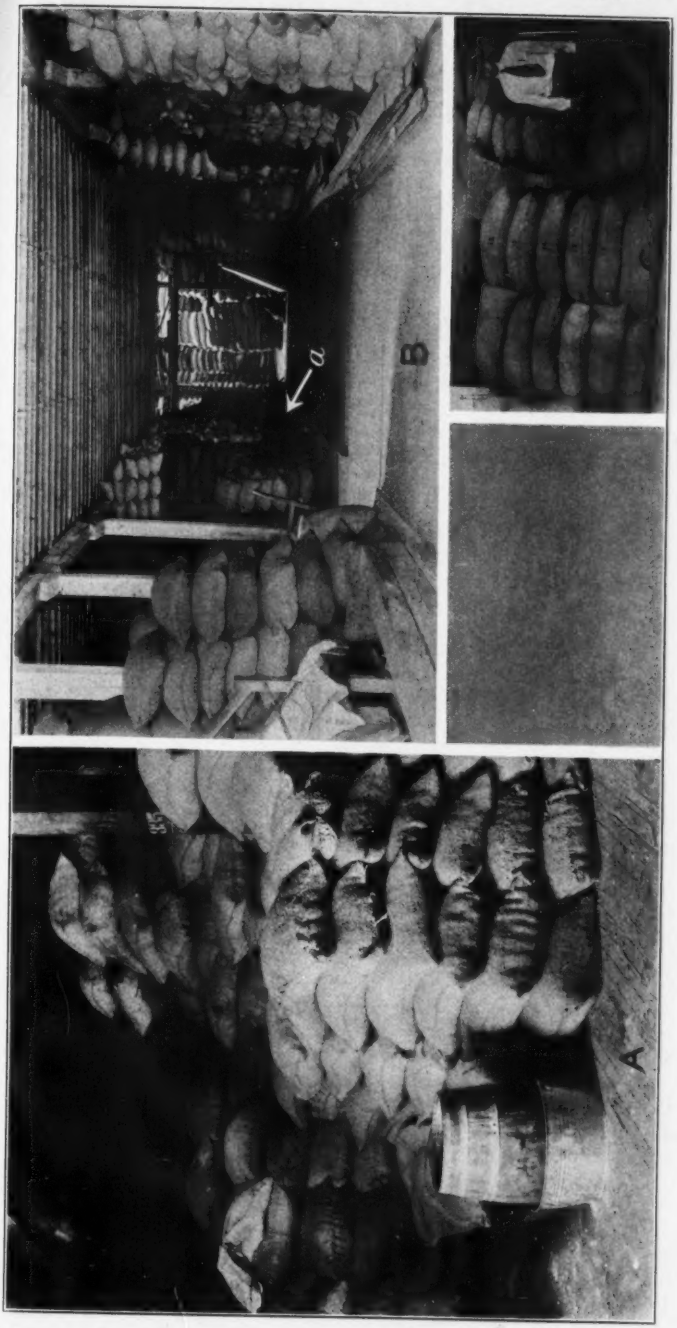
Since *Bruchus quadrimaculatus* is the primary pest of chick-peas, fumigation with hydrocyanic-acid gas, when generated by the use of 2½ pounds of 98 to 99 per cent pure sodium cyanid per thousand cubic feet of space, was shown to be a dependable method of protecting chick-peas in storage. Fumigation with hydrocyanic-acid gas has now become the standard control in commercial establishments handling this food.

PLATE 1

A.—Interior view of storage room of Warehouse No. 1. Note abnormal piling of sacks, making gas penetration more difficult, and barrel in tub used as generator for gas.

B.—Interior view of ground floor of Warehouse No. 3. Weevil infestations breaking out under such abnormal conditions were thoroughly controlled, even when the infestations were in sacks at *a* on the floor.

C.—Fairly normal method of storing chick-peas. Note carload lot to left, only two of the tiers of sacks showing, and narrow space between carload lots extending from main aisle to the rear. Man with grain car probe about to take samples from sacks No. 12 to 17, which are the same sacks as those shown in text Figure 1, *c*.


B
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A NEW NEMATODE, CYLINDROPHARYNX ORNATA, FROM THE ZEBRA, WITH KEYS TO RELATED NEMATODE PARASITES OF THE EQUIDAE [1]

By ELOISE B. CRAM

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Cylindropharynx ornata, sp. n.

Two males and one female of this species were found in material collected from *Equus grevyi* at Bethesda, Md., in 1907, one male among specimens collected from the feces by Dr. E. C. Schroeder and the other two among specimens collected post mortem by Dr. M. C. Hall. The worms were collected from one or both of two zebras presented by the King of Abyssinia to Ex-President Roosevelt and turned over by him to the Department of Agriculture.

Size: Male 9.9 mm. in length and 0.4 mm. greatest breadth. Female 10.0 mm. in length and 0.5 mm. greatest breadth.

HEAD—MOUTH COLLAR.—Depressed at margin; marked off from rest of the skin by a constriction; it is considerably higher dorsally and ventrally than laterally.

HEAD PAPILLAE.—Submedian papillae very striking in appearance, a distal leaf-shaped portion being separated by a constriction and bent inward from the tall digitiform proximal portion.

Lateral papillae not conspicuous; do not project beyond mouth opening; their extremities are trifurcated, as noted by Boulenger (*1*)[2] in *C. brevicauda*.

MOUTH CAPSULE.—This shows the great length characteristic of the genus; in the male 250μ, in the female 300μ long. The diameter of the anterior third of the capsule is markedly greater than that of the posterior two-thirds, giving the appearance of a bulbous swelling. In the male the diameter of the anterior portion was 140μ, of the posterior portion 83μ; in the female 166μ and 99μ respectively. The ratio of greatest width to length of capsule was therefore 1: 1.8 in both sexes. The capsule is lined with the usual transparent chitinous layer, which in the anterior bulbous portion projects into the lumen in what appears as a ring-like thickening. The walls of the mouth capsule are thick, their diameter being 11μ in the anterior portion and 16μ in the posterior portion.

DORSAL OESOPHAGEAL GUTTER.—This does not project into the buccal capsule.

LEAF CROWNS.—The external leaf crown in this species, as in the three previously described, consists of six elements, all clearly projecting beyond the mouth opening. The four occupying the submedian positions are delicate, relatively small, and sharply pointed leaves; the two which are situated laterally are much broader; their tips are bent toward the exterior. The inner surface of these leaves is grooved, giving the appearance of three ridges with two troughs between them; these are divergent toward the base of the leaf. Boulenger (*1*) noted that the corresponding leaves of *C. brevicauda* were notched in a similar manner and suggests that this may indicate that the leaf resulted from the fusion

[1] Received for publication Mar. 27, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 672.

of at least two elements. In the present species at least three elements would be indicated from the grooves. Situated in the space between the broad leaf and the corresponding lateral papilla are two accessory elements, which have not been described, from the other species of Cylindropharynx. These are very transparent and their structure is ascertained only with difficulty; their position and general shape are shown in Figures 1, 2, and 3. From the dorsal and ventral

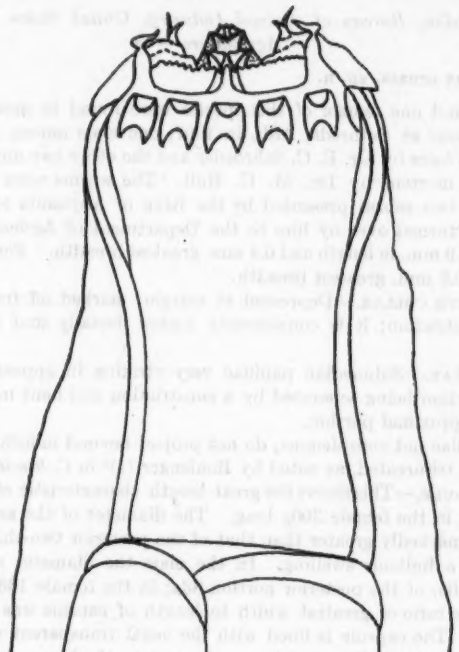

FIG. 1.—Anterior extremity in lateral view

lips of the mouth collar broad plates project into the lumen of the mouth. Their elaborate structure is shown in Figures 2 and 4. Similar plates have been noted by Yorke and Macfie (*8*) in *C. rhodesiensis* and *C. brevicauda*.

The internal leaf crown consists of 12 strikingly large, broad elements, the distal-free portion of each being bluntly rounded and bent inwards, and the basal attachment to the mouth capsule showing as a prominent crescentic line. A somewhat similar appearance of the attachment of the elements is seen in

Gyalocephalus capitatus in what Yorke and Maefie (7) regard as the posterior terminations of the internal leaf crown, but which Ihle (5) considers separate processes of the posterior border of the mouth capsule wall. These internal elements of *Cylindropharynx ornata* originate considerably behind the anterior margin of the mouth capsule, and on reaching that margin are bent inward as described above.

Oesophagus.—Short and broad, in the female having a length of 747μ and greatest breadth 216μ; in the male 581μ and 166μ respectively. The nerve ring is situated near the anterior extremity. The excretory pore lies at the union of the posterior and middle thirds of the oesophagus; the cervical papillae are situated a short distance anterior to the pore.

Intestine.—This shows pseudo-annulations throughout its length, which are especially striking in the anterior part. Leiper (4) indicated this in the figures of *C. brevicauda* and *C. longicauda*.

Posterior extremity of the male.—The breadth of the worm decreases only very slightly and the dorsal lobe of the bursa is short, so that the posterior end of the worm appears very blunt. The dorsal ray is 202μ long, tapers to a long, sharp point, and midway from base to tip gives off an external branch, which is bifurcated in its distal half, the inner finger-like process being slightly shorter than the other (fig. 5). There are no fine, hair-like projections on the dorsal rays such as are shown in figures of both *C. rhodesiensis* and *C. brevicauda*. The bursa is finely striated, its margin showing very fine serrations; the lateral lobes do not embrace the genital cone. The genital cone is short (100μ) and stout. The dermal collar is well developed on the ventral but not on the dorsal surface of the cone. The genital appendages are very striking and of very elaborate nature (fig. 6). A central portion, which lies directly under the lip of the genital cone, consists of two globular projections, each bearing a papilla-like point; these two are connected in the middle line by a thumb-like projection. On each side of these central structures there projects a large, irregular-shaped extension, its posterior line wavy, its free end a finger-like process, and on the anterior margin a knob-like projection. These two structures show variation in position, in one male being at right angles to the long axis of the cone (as figured) and in the other the angle being only half that. This variation may indicate that these are clasping organs, therefore motile and capable of changing their relative position. In addition to these complex appendages of the cone, the dermal collar bears an indefinite number of long, delicate, sharply pointed, flexible processes on its lateral and dorsal surfaces. The arrangement of these seems quite irregular. The spicules are long (790μ) and stout, their extremities sharply pointed and strongly barbed (fig. 7).

Posterior extremity of the female.—The vulva is 1411μ and the anus 330μ from the tip of the tail. Posterior to the anus there is an abrupt thickening, then the tail tapers to a sharp point. Covering the anus there is a lip-like structure of papilliform appearance (fig. 8).

Diagnosis.—This worm is more closely allied to *Cylindropharynx rhodesiensis* than to either of the other two species in the genus, but differs from it in its smaller size, in the much greater width of the mouth capsule in proportion to its length, in the point of origin of the internal leaf crown elements being considerably farther back and in their distal ends being bluntly rounded, in the presence of very elaborate genital appendages, and in the different shape of the spicules. A comparison of the four species of the genus is seen in the key on page 668.

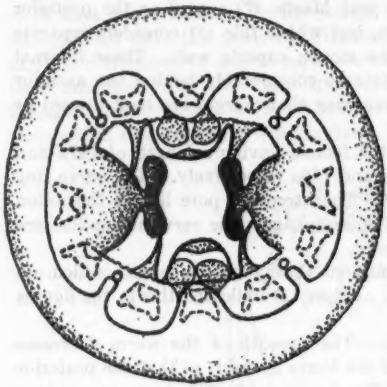

FIG. 2.—Head viewed from front

FIG. 3.—Broad external leaf-crown element—and secondary structures in front of it

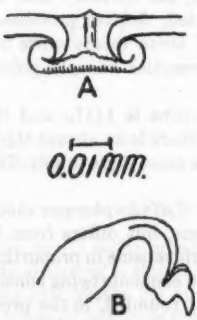

FIG. 4.—Horizontal plate of dorsal and ventral lips; (A) front view, (B) side view

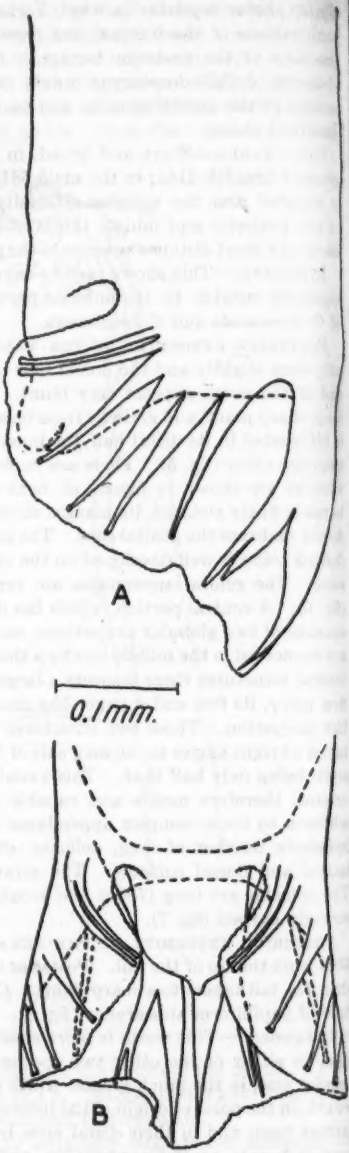

FIG. 5.—Bursa of male; A, lateral view; B, ventral view

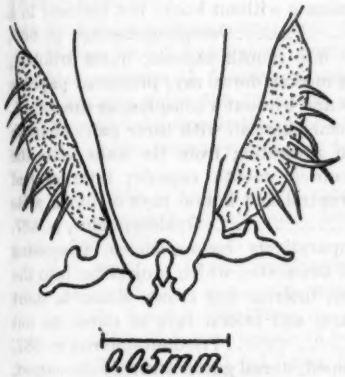

FIG. 6.—Appendages of genital cone and dermal collar

FIG. 7.—Spicule, lateral view

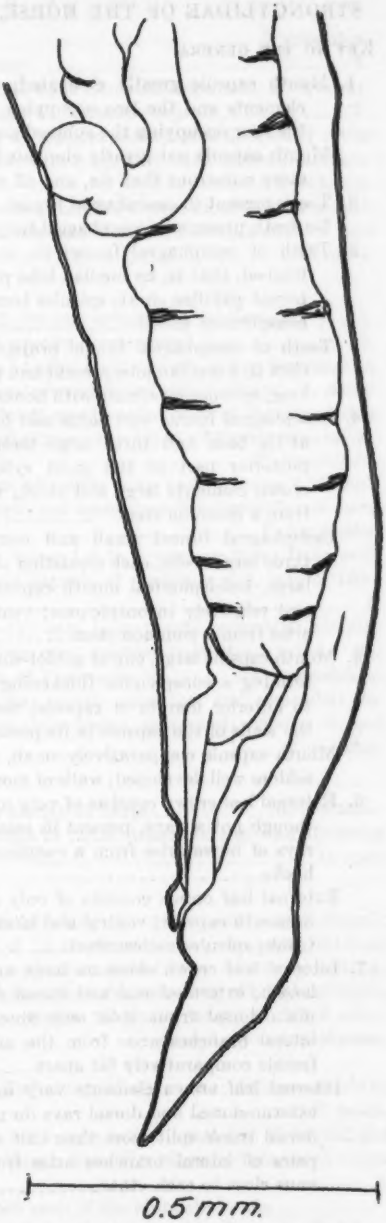

FIG. 8.—Posterior extremity of female, lateral view

STRONGYLIDAE OF THE HORSE, DONKEY, MULE, AND ZEBRA

KEY TO THE GENERA.

1. Mouth capsule greatly elongated; external leaf crown contains only six elements and the two occupying the lateral positions are broader than the four occupying the submedian positions---Cylindropharynx, p. 668.
   Mouth capsule not greatly elongated; external leaf crown elements usually more numerous than six, and all similar in size and shape---2.
2. Teeth present in oesophageal funnel---3.
   No teeth present in oesophageal funnel---5.
3. Teeth of oesophageal funnel do not project into mouth capsule; bursa bilobed, that is, no median lobe present, and no median dorsal ray; prebursal papillae short; spicules terminate without hooks but inclosed in a conspicuous sheath---Oesophagodontus, p. 667.
   Teeth of oesophageal funnel project into mouth capsule; bursa trilobed, that is, a median lobe present and a median dorsal ray; prebursal papillae long; spicules terminate with hooks and without a conspicuous sheath---4.
4. Oesophageal funnel very large and hemispherical, with three pairs of teeth at its base and three large teeth projecting from its walls into the posterior part of the short cylindrical mouth capsule; internal leaf crown elements large and stout; ventral and lateral rays of bursa arise from a common stem---Gyalocephalus, p. 667.
   Oesophageal funnel small and comparatively inconspicuous, possessing three large teeth, each consisting of two plates, which project far into the large, hemispherical mouth capsule; internal leaf crown elements short and relatively inconspicuous; ventral and lateral rays of bursa do not arise from a common stem---Triodontophorus, p. 667.
5. Mouth capsule large, cup or goblet-shaped; dorsal gutter strongly developed, forming a conspicuous thickening along dorsal wall almost or entirely to anterior margin of capsule; teeth may be present as projections of the walls of the capsule in its posterior part---6.
   Mouth capsule comparatively small, more or less cylindrical; dorsal gutter seldom well developed; walls of mouth capsule never give rise to teeth---7.
6. External leaf crown consists of very numerous slender elements; teeth often, though not always, present in mouth capsule; dorsal and externo-dorsal rays of bursa arise from a common trunk; spicules do not terminate in hooks---Strongylus, p. 667.
   External leaf crown consists of only a few large, broad elements; no teeth in mouth capsule; ventral and lateral rays of bursa arise from a common trunk; spicules undescribed---Craterostomum, p. 668.
7. Internal leaf crown elements large and broad, sometimes not all of equal length; externo-dorsal and dorsal rays arise from a common trunk; this main dorsal trunk split only about half-way to the base, so that the lateral branches arise from the undivided portion; vulva and anus of female comparatively far apart---Poteriostomum, p. 667.
   Internal leaf crown elements vary in size and shape in different species; externo-dorsal and dorsal rays do not arise from a common trunk; main dorsal trunk split more than half way to the base, so that one or both pairs of lateral branches arise from the divided portions; vulva and anus close to each other---Cylicostomum *sensu lato*, p. 668.

STRONGYLUS.

1. No teeth in mouth capsule ..... *S. edentatus.*
   One or more teeth in mouth capsule ..... 2.
2. Teeth on both the dorsal and ventral walls of mouth capsule .. *S. equinus.*
   Only one bifurcated tooth on dorsal wall of mouth capsule ..... 3.
3. Male not over 16, female not over 24 mm.; excretory pore at level of nerve ring; the two lobes of the tooth are high and have smooth margins. *S. vulgaris.*
   Male 18–32 mm., female 30–43 mm.; excretory pore close behind mouth collar; the two lobes of the tooth low and broad and margins divided into rounded cusps ..... *S. asini.*

TRIODONTOPHORUS.

1. Median lobe of bursa long; teeth of pharynx usually not denticulated ..... 2.
   Median lobe of bursa short; teeth usually denticulated ..... 3.
2. Male not over 13, female not over 14 mm. long; mouth collar depressed at margin; vulva 535–700μ from posterior end; tail region conical .. *T. minor*
   Male 13.5–14, female 16–17 mm. long; mouth collar erect at margin; vulva 270–300μ from posterior end; tail region very blunt, obliquely truncated. *T. brevicauda.*
3. Tail region of female short; vulva 460–560μ from end; spicules 1.25 mm. long; mouth capsule small, length not more than 90μ, breadth not more than 140μ ..... *T. tenuicollis.*
   Tail region of female elongated; vulva 1.4 to 2 mm. or more from end; spicules 3.5–4 mm. long; mouth capsule of moderate size, length 100–130μ, breadth 150–180μ ..... 4.
4. Male 14.5–15.5 mm., female 16.5–18.7 mm. long; mouth collar high, circular in profile; vulva 1.4–1.7 mm. from end; spicules have stout barbs. (G. Theiler says this is a synonym of *T. serratus.*) ..... *T. intermedius.*
   Male 18 mm., female 25 mm. long; mouth collar low and somewhat depressed at margin; vulva more than 2 mm. from end; spicules do not have barbs ..... *T. serratus.*

GYALOCEPHALUS.

1. Genital cone extends only to free margin of bursa ..... *G. capitatus.*
   Genital cone extends beyond margin of bursa; length of cone usually twice as great as preceding species ..... *G. equi.*

OESOPHAGODONTUS.

Males 15–16 mm., females 19–22 mm. long; mouth capsule goblet-shaped; external leaf crown composed of 18 large, projecting elements; internal leaf crown of 36–48 slender elements; dorsal lobe of bursa lacking; postero-lateral ray branched at base; posterior extremity of female straight, gradually tapering from vulva region to tip (a length of 2.3–3 mm.) *Oesophagodontus robustus.*

POTERIOSTOMUM.

1. All the elements of the internal leaf crown of equal size; distance of vulva from anus 925μ to 1.5 mm ..... *P. ratzii.*
   All the elements of the internal leaf crown not of equal size (2 lateral and 4 submedian elements being longer than the others); distance of vulva from anus 680 to 825μ ..... 2.
2. Seven small leaf elements between each of the elongated ones. *P. imparidentatum.*
   Ten small leaf elements between each of the elongated ones. *P. pluridentatum.*

CRATEROSTOMUM.

1. Length 9 mm. or more; internal leaf crown consisting of not more than 16 elements; oesophagus over 500μ long; vulva 1.27 mm. or more from tip of tail ..... *C. acuticaudatum.*
   Length not over 8 mm.; internal leaf crown consisting of 18 or more elements; oesophagus not over 400μ long; vulva not more than 1.1 mm. from tip of tail ..... 2.
2. Females 8 mm. long; internal leaf crown consisting of 24–25 elements; anus 425–535μ; vulva 905–1100μ from tip of tail ..... *C. mucronatum.*
   Females 4–5.5 mm. long; internal leaf crown consisting of 18 elements; anus 250–270μ, vulva 530–570μ, from tip of tail ..... *C. tenuicauda.*

CYLINDROPHARYNX.

1. Worms more than 12 mm. long; length of mouth capsule more than 3¼ times its greatest width; no genital appendages ..... *C. rhodesiensis.*
   Worms less than 12 mm. long; length of mouth capsule not greater than 3¼ times the greatest width; genital appendages present ..... 2.
2. Length of mouth capsule from 3 to 3¼ times its greatest width; genital cone long (up to 400μ), extending considerably beyond margin of bursa; vulva of female not more than 750μ, anus not more than 200μ from tip of tail ..... *C. brevicauda.*
   Length of mouth capsule less than 3 times its greatest breadth; genital cone short, not extending beyond margin of bursa; vulva of female more than 750μ, anus more than 200μ from tip of tail ..... 3.
3. Worms not over 7 mm. long; length of mouth capsule more than twice its greatest width; extremity of lateral branch of dorsal ray undivided or with only a very small projection; appendages a simple pair of stout, finger-shaped processes ..... *C. longicauda.*
   Worms over 7 mm. in length; length of mouth capsule less than twice its greatest width; extremity of lateral branch of dorsal ray deeply bifurcated; appendages elaborate structures on genital cone and on dermal collar ..... *C. ornata.*

CYLICOSTOMUM SENSU LATO.

Ihle (2) has divided the large number of species previously placed under Cylicostomum Railliet into seven groups, to five of which he gives the rank of subgenera. I prefer to raise these to genera and to give the two remaining groups generic names, believing that this will simplify matters. For the Montgomery group of Ihle I propose the name Cyclicotoichus, and for his Brevicapsulatum group the name Cylicobrachytus. The latter group contains only two species; further study may show that they do not belong in the same genus, but until that time confusion will be avoided by placing the group in a position corresponding with that of the other groups which were made by Ihle.

1. Dorsal and ventral walls of mouth capsule much higher (that is, longer in the direction of the long axis of the worm) than lateral walls. *Cylicotoichus.*
   Mouth capsule radially symmetrical (that is, wall of equal height throughout, or without great difference in height). (See *C. brevicapsulatum*) ..... 2.
2. Mouth capsule extremely short (may be as high as 13–14μ in *C. prionodes*); elements of internal leaf crown either inconspicuous or wanting. *Cylicobrachitus.*
   Mouth capsule not extremely short (may be as little as 17μ high in *C. labratum*) ..... 3.
3. Posterior end of female strongly bent dorsally, with a swelling before the vulva, the tail in lateral view resembling a human foot .. *Cylicocercus.*
   Posterior end of female straight or slightly bent dorsally ..... 4.

4. Internal leaf crown elements extremely large.....Cylicodontophorus.
Internal leaf crown elements small.....5.
5. Internal leaf crown elements originate at some distance behind anterior margin of the mouth capsule..... *Cylicostomum sensu stricto.*
Internal leaf crown elements originate close to anterior margin.....6.
6. Mouth capsule with hoop-like thickening at posterior margin. *Trichonema.*
No hoop-like thickening.....7.
7. Mouth capsule outline mostly rectangular or trapezium-shaped in lateral or dorso-ventral view; posterior end of female mostly straight. *Cylicostephanus.*

CYLICOTOICHUS.

Males 4.3–6 mm., females 4.5–6.5 mm. long; dorsal and ventral walls of mouth capsule 32μ, lateral walls 22μ high; external leaf crown consists of 18 unusually long pointed leaves; dorsal lobe of male bursa of medium length; tail of female very short and finely pointed; only one type species. *C. montgomeryi.*

CYLICOBRACHYTUS.

1. Male 11.5, female 13.5 mm. long; external leaf crown consists of 45 slender elements; oesophagus comparatively short (in female 635μ). *C. brevicapsulatum.*
2. Females only described, 10–11.5 mm. long; external leaf crown consists of 24–26 broad-based elements; oesophagus long (800–935μ); type of genus. *C. prionodes.*

CYLICOCERCUS.

1. Genital cone very long (about 664μ); elements of external leaf crown number about 48.....*C. mettami.*
Genital cone not elongated; elements of external leaf crown number not over 30.....2.
2. Three triangular teeth projecting into mouth capsule from walls of oesophageal funnel.....3.
No teeth projecting into mouth capsule.....4.
3. Appendages of genital cone absent, but posterior part of collar bears two sharply pointed processes directed toward each other...*C. tridentatum.*
Appendages of genital cone present as thin, delicate plates, with two slender, finger-like processes.....*C. goldi.*
4. Median lobe of bursa fairly long; elements of the external leaf crown very slightly if at all projecting out of mouth opening.....*C. alveatum.*
Median lobe of bursa extremely short, almost semicircular; elements of leaf crown clearly projecting out of mouth opening.....5.
5. Walls of mouth capsule (ventral view) have convex outer surface; inner surface undulating to give two notches, the posterior, which is the deeper, being the site of insertion of inner leaf crown; appendages of genital cone consist of slight elevations, with three conical processes, the innermost bearing a delicate finger-like projection..... *C. pateratum.*
Walls of mouth capsule (ventral view) curve concavely on outer surface, convexly on inner; appendages do not have three conical processes...6.
6. Appendages of genital cone moderately long, outer edges notched, ends rounded; no elongate or digitate processes..... *C. catinatum.*
Elongate or digitate processes on appendages of genital cone.....7.
7. Each appendage consists of two long, slender, finger-like processes, each with a short projection on its inner surface and with a short projection on each side between the two processes....*C. catinatum* var. *literaureum.*
Each appendage consists of a slight elevation, with two simple, unbranched conical processes..... *C. pseudocatinatum.*

CYLICODONTOPHORUS.

1. Internal leaf-crown possesses dissimilar elements, 12 of the 46 elements being enlarged and projecting farther than those of the external leaf crown. *C. ultrajectinum.*

   All elements of internal leaf crown similar ..... 2.

2. Dorsal gutter projects into mouth capsule, almost to its anterior border; median lobe of bursa elongated, triangular ..... *C. bicoronatum.*

   No dorsal gutter; median lobe of bursa of moderate size ..... 3.

3. Female 14 mm., male 9–10 mm.; outer leaf crown 60, inner 40–46 elements; posterior end of female suddenly falls away behind the anus to form a short tail; genital cone of male small ..... *C. ihlei.*

   Female 6–8, male 6–7 mm.; outer leaf crown 40, inner 30–34 elements; posterior end of female tapers very gradually to a point, the axis always remaining the same as that of body; genital cone of male relatively enormous ..... *C. euproctus.*

CYLICOSTOMUM sensu stricto.

1. Dorsal oesophageal gutter does not extend along wall of mouth capsule ... 2.

   Dorsal oesphageal gutter does extend along wall of mouth capsule ..... 3.

2. Mouth capsule short (17–18μ), wider than long, walls of moderate thickness; median lobe of bursa short and pointed ..... *C. labratum.*

   Mouth capsule fairly deep (33μ, Ihle says 37–41μ), longer than wide, walls remarkably thick; median lobe of bursa long and slender, its sides almost parallel ..... *C. coronatum.*

3. Walls of mouth capsule converge from before backwards, are irregular of outline, kneed at least twice on both inner and outer surface. *C. aegyptiacum.*

   Walls straight; outline not as irregular as above ..... 4.

4. Females 12–12.5, males 10–11 mm. long; internal leaf crown of 70–80 elements; median lobe of bursa long and slender ..... *C. sagittatum.*

   Females not over 11, males not over 9 mm.; internal leaf crown of not more than 50 elements; median lobe of bursa short ..... 5.

5. Elements of external leaf crown project $\frac{1}{5}$ their height; dorsal oesophageal gutter extends $\frac{1}{5}$ depth of capsule; lateral prominences of posterior extremity of female well developed ..... *C. ornatum.*

   Elements of external leaf crown only very slightly projecting; dorsal gutter forms short, blunt tubercle on floor of capsule; lateral prominences of posterior extremity of female not well developed ..... 6.

6. Appendages of male genital cone absent, replaced by two pointed prominences on posterior part of dermal collar; distance from vulva to anus of female 150μ ..... *C. labiatum.*

   Dermal collar of male has prominences like those of *C. labiatum*, but the posterior part of genital cone also has finger-like processes on both sides, varying in number and form; distance from vulva to anus of female 95μ. *C. labiatum* var. *digitatum.*

TRICHONEMA.

1. Males 16–17 mm., females up to 26 mm.; mouth collar strongly enlarged in lateral sectors, relatively low in dorsal and ventral sectors; excretory pore posterior to origin of intestine by a distance equivalent to half the length of the oesophagus ..... *T. auriculatum.*

   Males not over 14 mm.; females not over 17 mm.; mouth collar of approximately the same height in all sectors; excretory pore either at junction or anterior to junction of oesophagus and intestine ..... 2.

2. External leaf crown elements short and flat, their free extremities rounded and not extending to the anterior margin of the collar ..... *T. radiatum.*

External leaf crown elements not short and flat, their free extremities pointed and extending to or beyond the anterior margin of the collar. 3.

3. Dorsal gutter present ..... 4.
   Dorsal gutter not present ..... 6.
4. Internal leaf crown elements (52 in number) quite large and broad; dorsal gutter short, just extending above base of capsule; prebursal papillae very long (250μ) ..... *T. adersi.*
   Internal leaf crown elements minute plates; dorsal gutter well developed, terminating about midway between anterior and posterior margin of capsule; prebursal papillae short (not more than 70μ) ..... 5.
5. Males 8–10 mm.; females 9–14 mm; accessory rays of bursa vary. *T. nassatum.*
   Males 6.5–7.5, females 8.2–9.7; median dorsal ray of bursa has constant accessory side branch ..... *C. nassatum* var. *parvum.*
6. Excretory pore at junction of oesophagus and intestine ..... *T. tetracanthum.*
   Excretory pore anterior to junction of oesophagus and intestine ..... 7.
7. Males 6, females 7–9 mm.; external leaf crown consists of 20–24 elements; buccal capsule 27μ deep; distance between anus and vulva 81μ. *T. leptostomum.*
   Males 12.3–13, females 12.3–17 mm.; external leaf crown consists of 30 or more elements; buccal capsule 38μ or more deep; distance between anus and vulva 180μ or more ..... 8.
8. Males and females 12.3 mm.; external leaf crown elements project only very slightly from mouth opening and their free extremities are drawn in toward each other; median lobe of bursa of medium length (posterior ray 635μ) and fairly broad ..... *T. triramosum.*
   Males 13, females 17 mm.; external leaf crown elements project considerably out of mouth opening and curve outward; median lobe of bursa long (posterior ray 847μ or more) ..... 9.
9. Posterior ray of median lobe of bursa about 847μ ..... *T. elongatum.*
   Posterior ray of median lobe of bursa about 1.5 mm. *T. elongatum* var. *kollani.*

CYLICOSTEPHANUS.

1. External leaf crown consists of not more than 8 elements; dorsal gutter extends along the mouth capsule almost to anterior margin ..... 2.
   External leaf crown consists of from 14–35 elements; dorsal gutter projects only slightly or not at all along mouth capsule ..... 3.
2. Males 6–6.5, females 8 mm. long; oesophagus shows well-marked, ring-like enlargement at its anterior end; median lobe of bursa narrow and long (dorsal ray about 525μ); posterior extremity of female straight. *C. calicatum.*
   Males 4–4.6 mm., females 4.1–5.6 mm. long; oesophagus does not show ring-like enlargement at anterior end; median lobe of bursa broad and short (dorsal ray 140–198μ); posterior extremity of female usually has S-shaped bend ..... *C. minutum.*
3. Mouth capsule very deep (about 70μ); external leaf crown consists of about 35 elements; oesophagus very long (about 0.8 mm.), tail of female long (anus 0.3 mm. from tip) ..... *C. poculatum.*
   Mouth capsule comparatively shallow (17–32μ); external leaf crown consists of 14–18 elements; oesophagus not elongated (0.2 to 0.4 mm.); tail of female comparatively short (anus 0.05–0.1 mm. from tip) ..... 4.
4. Males 4.8–5.5 mm., females 4.7–5.7 mm. long; dorsal lobe of male bursa narrow, tapering and very long; anus of female 95–124μ from tip of tail ..... *C. longibursatum.*
   Males 9–9.5 mm., females 10–10.5 mm. long; dorsal lobe of male bursa short and broad; anus of female 56μ from tip of tail ..... *C. hybridum.*

ADDENDUM

Since the writing of this paper Railliet (*5*) has published a description of *Strongylus tetracanthus* Mehlis and has shown that it is identical with *Trichonema arcuata* Cobbold and with *Cylicostomum insigne* Boulenger. The name of the species therefore becomes *Trichonema tetracanthum* (Mehlis, 1831) Railliet and Henry, 1919. *Cyathostomum tetracanthum* Looss is a different species and Railliet has named it *Trichonema aegyptiacum*.

Since in raising Ihle's subgenera to genera, *C. insigne* Boulenger was referred to the genus Cylicocylus, the latter will fall as a synonym of Trichonema. Cylicostomum, with the type *C. aegyptiacum*, is therefore available as a generic name for species of the restricted genus Cylicostomum as used in this paper, and equivalent to the subgenus Cylicostomum as used by Ihle.

Railliet (*6*) has divided the genus Strongylus into four subgenera. One of these, Decrusia, contains the strongyle of the elephant. The three subgenera of the horse strongyles, and the distinguishing characteristic of each are as follows: Strongylus, two subventral and two dorsal teeth; Alfortia, no dorsal teeth; Delafondia, two dorsal teeth.

Accordingly the four species are *Strongylus equinus*, *Str.* (*Alfortia*) *edentatus*, *Str.* (*Delafondia*) *vulgaris*, *Str.* (*Delafondia*) *asini*.

Gertrud Theiler in a thesis published in 1923, which has just come to hand, has described a new species of Cylindropharynx, *C. intermedia*, from the South African Zebra. It is distinguishable from the other four species as given in the key in this paper in that the specimens of *C. intermedia* are less than 12 mm. long and the length of the mouth capsule is 4 to 5 times as great as its greatest width.

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CHROMOSOMES IN MAIZE AND MAIZE RELATIVES [1]

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An understanding of the laws of heredity is essential if improvement of plant breeding is to be controlled intelligently. A comprehensive understanding of the mechanism of inheritance demands definite knowledge of the chromosome number, not only of the forms it is desirous to improve but also of the related species. The present paper presents the results of a study of the chromosome number in several genera of the tribe Tripsaceae made in connection with genetic studies of *Zea mays* and its relatives.

Very early in the investigation it was found that *Euchlaena perennis* had 20 pairs of chromosomes instead of 10, the number found in annual teosinte, *E. mexicana*, and in maize. Since perennial teosinte hybridizes with maize and the cross seems likely to be of much genetic interest, it seems desirable to publish this fact together with other cytological data which have come to light.

The larger part of the material used in this study was from plants growing during the summer of 1923 at Lanham, Md., Arlington, Va., and in one of the department greenhouses at Washington, D. C. The material for the study of the chromosomes of two Mexican Tripsacums was collected in Mexico by Messrs. Collins and Kempton the same season.

Two cytological methods of investigation were used in the study of pollen mother-cell development. The iron-acetic-carmine method outlined by Belling (*1*)[2] was used while growing material was available and it was found that very splendid mitotic figures were quickly stained and ready for study. To verify the results of this quick method, material was also killed in chromo-acetic or Bouin's solutions, embedded in paraffin, sectioned, and stained in Heidenhain's iron-alum haematoxylin. The former method is very satisfactory for studying chromosomes, for they appear more normal than in material prepared by the second method, but the second method has the advantage of leaving permanent slides as records of the conditions found.

EUCHLAENA

Euchlaena perennis Hitch. Perennial teosinte.

The material for a cytological study of the pollen mother-cell development of this species was taken from plants collected or from plants grown from seed collected in Mexico at the same time by Collins and Kempton (*7*). These plants were in one of the department greenhouses, Washington, D. C., and at Lanham, Md. The latter had been transplanted from the greenhouse in April. No difference was apparent in materials from plants growing at these two localities.

The haploid chromosome number in *E. perennis* was found to be 20 (Pl. 1, A and B). Since this species is likely to become more important as it becomes better known by corn experimenters, great care was taken in determining the number and size of the chromosomes. The 20 bivalent chromosomes, as represented in Plate 1, C, are distributed in a regular manner during the heterotypic division.

[1] Received for publication Apr. 9, 1924.

[2] Reference is made by number (italic) to "Literature cited," pp. 680-681.

E. mexicana Schrad. Annual teosinte.

Favorable pollen mother-cells of this species show 10 as the haploid chromosome number (Pl. 2, B, E, and K), corresponding to the number determined by Kuwada (*14, 15*). Plate 2, H, represents a heterotypic anaphase with 11 chromosomes going to each pole. This number was found in a very small per cent of the cells studied. In the few anaphases found that had more than 10 divided chromosomes the extra chromosome was slow in dividing and the halves lagged in their movement to the poles.

It was found in material collected for this study that the pollen mother-cells were very frequently abnormal in appearance; the mother-cell tissue of an anther was in one mass, with the nuclei dotted here and there through it. This mother-cell mass eventually subdivided into pollen grains, dividing first into polynucleated sections and finally into cells with a single nucleus. It was found that these nuclei contained the reduced number of chromosomes, but it was not determined whether such cells developed into normal pollen grains or not. Kuwada (*13*) described a very similar abnormality in amber rice popcorn. He is inclined to think that this was due to insufficient nutrition. The author will not attempt an explanation until material grown under more favorable climatic conditions has been studied.

Zea mays L.

To the present time four varieties of corn have been examined. All show 10 bivalent chromosomes at diakinesis. Plate 2, D, represents a typical heterotypic metaphase in the pollen mother-cell development of Chinese waxy maize, and Plate 2, A, the same phase in Tepic corn, a very large variety introduced from Mexico.

The author's investigations of corn have failed to show any deviation from 10 as the haploid chromosome number. The presence of 20 chromosomes in perennial teosinte and of 10 in annual teosinte and waxy and starchy corns makes it appear probable that the basic chromosome number in Euchlaena and Zea is 10. This view opposes that suggested by Kuwada (*13*), that is, that the number 12 is original for all the races of *Zea mays* and that *Zea mays* is tetraploid and derived from an original form which had probably 6 chromosomes in reduced numbers.

EUCHLAENA × ZEA

Zea mays × E. mexicana (F_2).

The material for this study was taken from a normal and a dwarf plant in an F_2 progeny of a cross between *E. mexicana* from Chalco, Mexico, and maize of the type known as dwarf (*10*). The chromosome number in each plant is 10, as was determined by Kuwada for a *Euchlaena-Zea* hybrid (*14, 15*). No irregularity in chromosome distribution was observed in the pollen mother-cell development.

Zea mays × E. perennis.

A dozen or more of these hybrids had been made in 1922, and a morphological and cytological study was made of the pollen development in F_1 plants of nearly all of these. Table I shows pollen counts of mature pollen from seven representative F_1 hybrids. These counts were made from pollen stained with chloral-hydrate-iodin solution (water 50 cc., chloral hydrate 50 gm., iodin 1 gm., and absolute alcohol 50 cc.). The pollen was taken from anthers just before they opened. At this time grains containing protoplasm, and therefore seemingly viable, stained deep blue or red, due to the presence of starch or erythrodextrin,

while all grains without protoplasm, and therefore sterile, remained unstained. The table also summarizes the chromosome condition found in 20 heterotypic prophases of 9 of these hybrid forms.

TABLE I.—*Records of pollen and pollen formation studies in Euchlaena perennis × Zea mays*

Plant name	Plant No.	Pollen counts			Heterotypic prophase chromosome condition			Remarks
		Fertile		Sterile				
		Blue	Red	Clear	Trivalent	Bivalent	Univalent	
Z. mays x *E. perennis:*								
Female parent—								
Oregon Evergreen	Th. 102	290		330				
S. P. I. 51291	Th. 103	175		356				
Missouri Cob Pipe	Th. 79				4	7	6	
Do	Th. 79	118		114	4	6	6	
Crinkly	Th. 82	69		119	3	7	7	
E. perennis x *Z. mays:*								
Male parent—								
Brachytic crinkly tassel seed	Th. 87	174		121	1	9	9	
Do	Th. 87	174		121	1	9	9	
Pod	Th. 88				3	7	7	
Do	Th. 88				3	7	7	
Do	Th. 88				3	7	7	
Do	Th. 88				4	6	6	
Do	Th. 88				5	5	5	
Do	Th. 88				1	9	8	Shows only 29 units.
Crinkly lineate	Th. 92	100		209	2	10	4	
Do	Th. 92	100		209	2	10	4	
Dwarf	Th. 93				2	3	18	Early prophase.
Brachytic dwarf	Th. 94				4	5	8	
Brachytic liguleless ramose	Th. 95				3	9	3	
Do	Th. 95				4	7	4	
Do	Th. 95				7	4	1	
Chinese waxy	Th. 96	296	13	96		10	10	
Do	Th. 96	296	13	96	2	9	6	

A study of the pollen mother-cell development in these hybrids showed that the attempt to combine the 10 chromosomes from the corn parent and 20 chromosomes from the teosinte parent gave varying counts. The author for some time found it difficult to determine the chromosome number. The difficulty was due to an irregularity in chromosome fusion and also to the fact that the chromosome number for *Euchlaena perennis* had not been determined at this time. Finally it was found that there are three classes of chromosomes at diakinesis (Pl. 1, E, F, and G), trivalents, bivalents, and univalents. It required practice and good material to segregate the chromosomes according to the above classes, but counts at diakinesis and the heterotypic metaphases gave uniformly 30 chromosome units.

Table I shows the condition found in 20 nuclei selected from different hybrids. The figures are taken from drawings made of characteristic and clear heterotypic phases. A minute study of trivalent chromosomes at this stage shows that many have a large part corresponding to a bivalent chromosome and a small, loosely attached portion. In a few cases the trivalent chromosomes seem to be composed of three loosely and temporarily attached chromosomes. The author considers that these loosely attached chromosomes are univalents which for a short period unite with bivalents or with each other to give trivalent or perhaps bivalent chromosomes.

It is 10 univalent chromosomes that seem to cause the irregularities observed in the fusing of the chromosomes, and in their distribution during the reduction divisions. The heterotypic (Pl. 1, H and I) and to a less degree the homotypic (Pl. 1, J) divisions are very characteristic. The bivalent chromosomes split in a normal manner and move toward the poles. This division seems also to include all or nearly all of the trivalent chromosomes, one part of each going toward one pole and two parts toward the other, although the double portion appears to separate at about the time the chromosomes move from the plate; consequently all move as single units to the poles. The univalent chromosomes are found scattered a bit apart from the nuclear plate while the bivalent and trivalent chromosomes are dividing. Following the separation of the bivalents and trivalents the univalents collect at the nuclear plate (Pl. 1, G). By the time the earlier-divided chromosomes arrive at the poles the univalents at the plate move, some toward the one, some toward the other pole (Pl. 1, H), without undergoing division. These undivided univalent chromosomes are frequently too late in their movement toward the poles to be included in the forming daughter nuclei and are left in the cytoplasm to degenerate (Pl. 1, I). A study of the homotypic divisions shows that occasionally chromosomes lag on the spindle, and some of them fail to be included in the daughter nuclei (Pl. 1, K). The outcome of such a meiosis is the very unequal distribution of the 30 chromosome units, seen at the heterotypic prophase, into the four resulting pollen grains.

This type of meiosis resembles that described by Rosenberg (*19*), Kihara (*12*), and other authors for hybrids resulting from crosses between species differing in chromosome number.

That the 10 univalent chromosomes, which lag on the spindle of the heterotypic and homotypic anaphases, came from the corn parent is suggested by the pollen study of one of these bigeneric hybrids. The study was made after it had been observed that the hybrids between starchy and waxy strains of maize, as well as hybrids between *Euchlaena mexicana* and waxy maize, produced pollen grains filled with starch and erythrodextrin in a 1:1 ratio. (See Table II.) The pollen of *E. perennis* × Chinese waxy maize showed only 3 or 4 per cent of the grains filled with erythrodextrin. If the 10 corn chromosomes had gone to make up the bivalent chromosome, approximately a 1:1 ratio of pollen grains filled with starch and erythrodextrin would have been expected. But if the 10 corn chromosomes are the univalents that lag on the spindle of the heterotypic and homotypic divisions and some of them fail to be included in the daughter nuclei, the result would be that a small per cent of the pollen would carry chromosomes having the erythrodextrin factor introduced by the corn parent.

TABLE II.—*Pollen counts of forms segregating for the starchy and erythrodextrin characters in pollen formation*

Plant	Fertile		Sterile	Per cent of red in fertile	Per cent sterile
	Blue	Red	Clear		
Starchy × waxy maize	2,069	2,046	A few.	49.7	
Waxy maize × *E. mexicana*	421	392	164	48.2	16.8
Waxy maize × *E. perennis*	296	13	96	4.2	23.7
Starchy coix × waxy coix	668	664	472	50.2	26.3
S. P. I. 48866	277	290	No count.	51.1	
Starchy coix × waxy coix	127	402	No count.	76.0	
S. P. I. 48867	521	1,310	2,582	71.5	58.5

Coix lachryma jobi L.

Table II also shows interesting pollen counts in two coix plants the pollen of which was segregating for the waxy-starchy character. The most significant thing noticed in these counts was the condition in S. P. I. No. 48867 where the number of grains showing erythrodextrin was double that showing starch. The high per cent of sterility allows for a 1:1 segregation of waxy and starchy grains, but the death rate in starchy grains must have been much higher than in the waxy. The presence of such a large per cent of erythrodextrin grains suggested the possibility that the segregation had been other than a 1:1 ratio as might occur if there were two factors controlling the expression of the character.

The chromosome number was determined for S. P. I. No. 48867, the coix plant showing the unexpected high per cent of grains staining red. The haploid number is clearly 10 (Pl. 2, C, F, G, I, and L). This number had previously been determined for coix by Kuwada (*14*, *15*).

Plate 2, J, was prepared to show the similarity between the 10 chromosomes of the two maize varieties studied and coix. This comparison of the chromosomes of maize and coix is introduced to show that the variation in the size of the individual chromosomes of coix are as great as those in maize. Kuwada (*13*) considers that a possible origin of the two large chromosomes in all 10-chromosomed corns is through a fusion of two chromosomes derived from a 12-chromosomed ancestor. The writer is of the opinion that 10 is the basic chromosome number for the Tripsaceae, and this view is supported by the similarity between the 10 chromosomes of corn and the 10 chromosomes of coix.

Tripsacum.

Tripsacum is less closely related to either Zea or Euchlaena than these two genera are to each other. Attempts to cross Zea and Tripsacum (*8*) have failed thus far. This investigation has revealed a very different chromosome complex in Tripsacum from that found in corn or in teosinte.

The haploid chromosome number for all Tripsacums examined appears to be 35. Plate 3, C and D, are drawings of a nucleus from *T. dactyloides*. The chromosomes were separated in about two equal portions by the microtome knife, the two sections showing 35 bivalent chromosomes. It is assumed that the fragment at the right in Plate 3, C, was cut from one of the chromosomes of Plate 3, D. Plate 3, E and F, represent the equal distribution of the chromosomes in the pollen mother-cell divisions. Such divisions are characteristic for all *T. dactyloides* material studied.

Plate 3, A and B, represents the condition in two phases of the heterotypic division of pollen mother-cells from *T. laxum* growing at the department greenhouse, Washington, D. C. These plants were grown from seed collected in Salvador. There are 36 chromosomes in the diakinesis pictured, but two of these have the appearance of univalents. The heterotypic anaphase pictured shows the irregular division of the chromosomes; 30 bivalent chromosomes have divided and are at the poles, while 10 univalent chromosomes are at the nuclear plate region in the process of division. The division of lagging univalent chromosomes at this time makes it necessary to study the early reduction phases in order to determine the chromosome number for forms characterized by such a division.

Other drawings (Pl. 3, H and I) show irregularities in the pollen formation of two Mexican Tripsacums. The cytological material for a study of these two forms was collected in Mexico by Messrs. Collins and Kempton in the fall of 1923 These figures demonstrate that the irregularities in chromosome distribution during meiosis are not due to greenhouse conditions.

Chromosome counts from pollen mother-cells of all species of Tripsacum studied are difficult and extremely so in all except *T. dactyloides*, where the reduction phases are more regular. The author attributes this difficulty in determining the chromosome number either to a lack of fusion of some chromosomes at diakinesis, giving nuclei that have more than 35 chromosomes, or to the combining of more than two chromosomes forming large chromosomes, giving nuclei with less than 35 chromosomes. The chromosome number, however, is too large to allow exact chromosome counts and classifications. Nevertheless a detailed study of pollen mother-cells of all the Tripsacum material available in the season of 1923 indicates that the haploid chromosome number is about 35.

DISCUSSION

The haploid chromosome number for representative species of the outstanding genera of the tribes of Tripsaceae (*15*) and Andropogoneae (*3*, *14*, *15*) have, with few exceptions, been found to be 10 and multiples of 10.

Kuwada (*13*) has used the chromosome number found in a few *Zea mays* strains as a basic for his view that Zea is a tetraploid species derived from ancestral types having 6 as the basic chromosome number. The author has made a preliminary investigation of the chromosome number in maize and maize relatives. He concludes, from the general occurrence of 10 and multiples of 10 chromosomes in the four genera of the Tripsaceae and from the presence of 20 haploid chromosomes in a recently discovered species, *Euchlaena perennis*, that 10 is the basic chromosome number for *Zea mays* and its relatives.

The outstanding species of this study has been *Euchlaena perennis*. The presence of 20 as the reduced chromosome number seems to attract attention toward the supposed close relation of the Euchlaenas and Zea. A chromosome number for perennial teosinte above that of the annual teosinte and maize makes the apparent relation among the three species difficult to explain. Morphological characters indicate that the annual teosinte and maize are of more recent origin than *E. perennis* and that Tripsacum is the least specialized member of this group of grasses. Chromosome numbers of typical representatives of these three genera indicate that the more primitive and less specialized members have more chromosomes than the more recent and highly specialized species.

The recent cytological studies in Rosa (*3*, *18*, *22*), Rubus (*16*), Oenothera (*6*), Datura (*4*), Triticum (*21*), Avina (*11*), Hyacinthus (*17*), and other polymorphic genera seem in a general manner to indicate that the more primitive and more stable species have a smaller chromosome number than more recent or hybrid forms. Such a conception must be reversed in order to derive *Zea mays* and *Euchlaena mexicana* from *E. perennis* or an *E. perennis*-like form having more than 10 haploid chromosomes, and to connect all these forms with the more primitive Tripsacums.

A critical investigation of the above-mentioned cytological studies shows very few hybrids that have a chromosome number differing from the sum of the haploid chromosomes of its parents, and only one case, a haploid Datura (*4*), in which there was a chromosome number smaller than that of its parent.

Rosenberg (*20*) utilized the occurrence of irregularities in chromosome distribution at meiosis to explain the increase in chromosome number of recently originated Crepis forms. This explanation generally applied to other genera should allow for an equal chance that hybrids will be found with a lower as frequently as with a higher chromosome number.

F_1 hybrids between maize and perennial teosinte have 30 chromosomes and at meiosis show a chromosome behavior such as has been generally described for

hybrids resulting from crosses between plants having different chromosome numbers.

The presence of trivalent chromosomes at diakinesis of the pollen mother-cell recalls the studies of Belling (*2*), in which he reports that, in polyploid forms, the chromosomes combine not only to form bivalent, but also trivalent, tetravalent, and even pentivalent groups. The random distribution of the undivided univalent chromosomes at the heterotypic anaphase of the pollen mother-cell development in this corn-teosinte hybrid is similar to that described by Sax (*21*) for the F_1 hybrids of *Triticum monococcum* × *T. turgidum*.

The possible identification of the univalent chromosomes present at meiosis in *E. perennis* × *Zea mays* hybrids is suggested by a study of the pollen where the corn involved was of the Chinese waxy type. The pollen in this cross had only 4 per cent of the grains showing erythrodextrin. There is also a very small percentage of meioses in which there are no extruded chromosomes. This may indicate that the extruded chromosomes are contributed to the hybrid by the waxy corn parent. This is contradictory to the chromosome behavior in the pollen mother-cells of a Nicotiana hybrid described by Goodspeed (*9*), where the bivalents were probably made up by a union of chromosomes from the two parents and the univalents were the leftovers from the parent with the larger chromosome number. The division of the univalent chromosome at the heterotypic division in this Nicotiana hybrid is unlike that found in teosinte-corn hybrids and similar to that described by Sax (*21*) for hybrids between the emmer and the vulgare groups of Triticum.

The study of the Tripsacums has shown that in this genus the haploid chromosome number is approximately 35. The reduction divisions of all Mexican forms studied resemble minutely that described by Bremer (*5*) for several varieties of *Saccharum officinarum*. In the Tripsacums, as in *S. officinarum*, chromosomes failed to fuse at diakinesis and the resulting univalents lag on the spindle. The division of lagging univalents occurs in the heterotypic division and this associated with a varying number of univalents in different pollen mother-cells makes exact chromosome counts very difficult.

SUMMARY

The chromosomes of *Euchlaena perennis* pair promptly at diakinesis of the pollen mother-cell, giving 20 bivalents.

The chromosome number of *Euchlaena mexicana* at diakinesis of normal-appearing pollen mother-cells is 10 bivalents. Occasionally 11 or 12 chromosomes have been found in reduction phases that appeared abnormal. The mother-cell tissue in anthers of this species is frequently in a multinucleated mass and very abnormal in appearance.

The chromosomes of *Zea mays*, strains Tepic and Chinese waxy, pair promptly at diakinesis of the pollen mother-cell, giving 10 bivalents.

The chromosomes of *Zea mays* × *Euchlaena mexicana* pair promptly at diakinesis of the pollen mother cell, giving 10 bivalents.

The chromosomes of *Zea mays* × *Euchlaena perennis* combine at diakinesis of the pollen mother-cell into trivalent or bivalent chromosomes or remain uncombined as univalents. The number of chromosome elements is 30, the sum of the haploid number of the two parents. The distribution of the 30 chromosome elements at meiosis is very irregular.

The chromosomes of *Coix lachryma jobi* pair promptly at diakinesis of the pollen mother-cell, giving 10 bivalents.

The chromosomes of *Tripsacum dactyloides* pair promptly at diakinesis of the pollen mother-cell, giving approximately 35 bivalents.

In *Tripsacum laxum*, *T. pilosum* and *T. lanceolatum* the presence of both univalent and bivalent chromosomes at diakinesis makes the assigning of a definite haploid chromosome number difficult. This number, nevertheless, is approximately 35.

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PLATE 1 [a]

A.—*Euchlaena perennis* Hitch. Heterotypic prophase, 19 bivalent and 2 single chromosomes.

B.—*Euchlaena perennis* Hitch. Heterotypic metaphase.

C.—*Euchlaena perennis* Hitch. Heterotypic anaphase.

D.—*Euchlaena perennis* Hitch. Early homotypic metaphase.

E.—*Euchlaena perennis* × *Zea mays*. Th. 9522. Heterotypic prophase, 7 trivalent, 4 bivalent, and 1 univalent chromosome.

F.—*Euchlaena perennis* × *Zea mays*. Th. 9522. Heterotypic metaphase, 3 trivalent, 8 bivalent, and 5 univalent chromosomes.

G.—*Zea mays* × *Euchlaena perennis*. Th. 10322. Heterotypic metaphase, 2 trivalent, 11 bivalent, and 2 univalent chromosomes.

H.—*Euchlaena perennis* × *Zea mays*. Th. 9322. Heterotypic anaphase; a typical irregular chromosome distribution.

I.—*Euchlaena perennis* × *Zea mays* (pod). Th. 9022. Late heterotypic anaphase.

J.—*Zea mays* × *Euchlaena perennis*. Th. 10322. Interkinesis showing two major nuclei and two minor nuclei.

K.—*Zea mays* × *Euchlaena perennis*. Th. 10222. Homotypic divisions.

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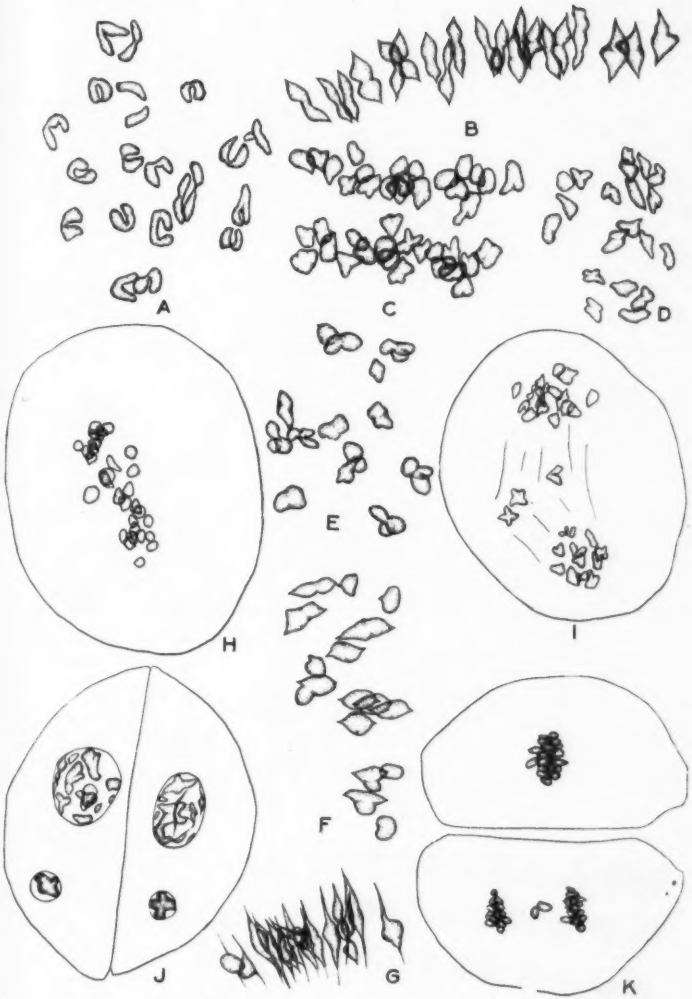
[a] The plates reproduce camera-lucida drawings of different phases in the reduction divisions of pollen mother-cells of several representative Tripsaceae. A 1.7 mm. oil immersion objective, a No. 12 compensating ocular, and cells stained with iron-acetic-carmine were used in preparing these drawings, except as follows: Heidenhain's iron-alum haematoxylin was used for staining Pl. 1, G to K; Pl. 2, A, D, G; Pl. 3, C and D, E to I. An ocular No. 18 was used for Pl. 2, A, D, G, and for Pl. 3, A. So far as possible, the drawings give details in size and shape of the individual chromosomes, thus allowing a comparison of chromosomes in any particular species or between closely related species. (Reduced one-third in reproduction.)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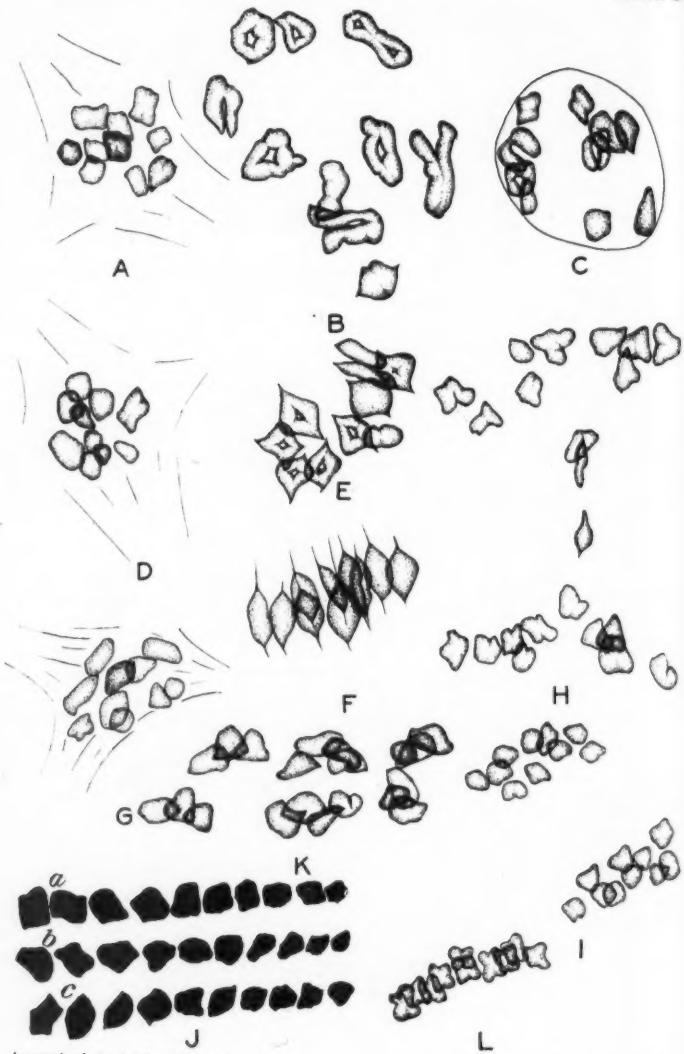
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PLATE 2

A.—*Zea mays* L. Tepic. Late heterotypic prophase.
B.—*Euchlaena mexicana* Schrad. Heterotypic prophase.
C.—*Coix lachryma jobi* L. S. P. I. No. 48867. Heterotypic prophase.
D.—*Zea mays* L. Chinese waxy. Late heterotypic prophase.
E.—*Euchlaena mexicana* Schrad. Heretotypic metaphase.
F.—*Coix lachryma jobi* L. S. P. I. No. 48862. Heterotypic metaphase.
G.—*Coix lachryma jobi* L. S. P. I. No. 48867. Late heterotypic prophase.
H.—*Euchlaena mexicana* Schrad. Heterotypic anaphase.
I.—*Coix lachryma jobi* L. S. P. I. No. 48867. Heterotypic anaphase with an extra chromosome lagging in its division.
J.—a. The 10 bivalent chromosomes from Pl. 2, A, arranged according to size.
b. The 10 bivalent chromosomes from Pl. 2, D, arranged according to size.
c. The 10 bivalent chromosomes from Pl. 2, G, arranged according to size.
K.—*Euchlaena mexicana* Schrad. Early heterotypic anaphase.
L.—*Coix lachryma jobi* L. S. P. I. No. 48862. Homotypic metaphase.

PLATE 3

A.—*Tripsacum laxum* Nash. Heterotypic prophase, showing 36 chromosomes, 2 of which are probably univalent.

B.—*Tripsacum laxum* Nash. Heterotypic anaphase, having the division of both bivalent and the smaller lagging probably univalent chromosomes.

C and D.—*Tripsacum dactyloides* L. Heterotypic prophase showing 35 bivalent chromosomes.

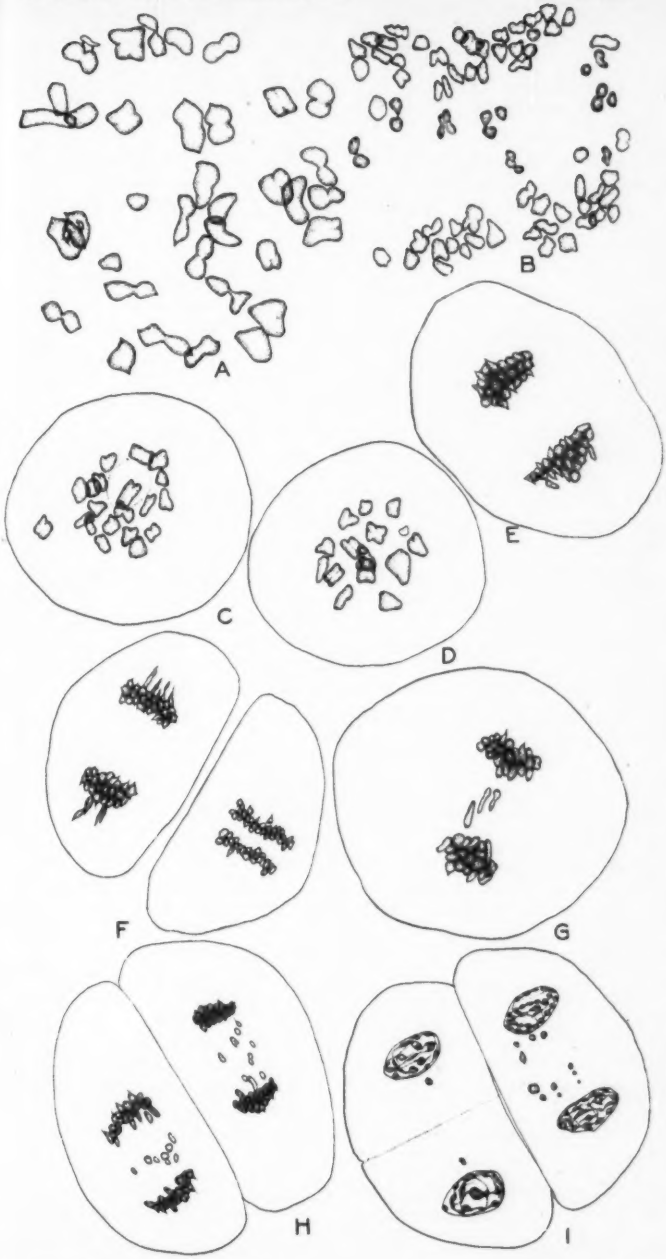
E.—*Tripsacum dactyloides* L. Heterotypic anaphase, showing regular distribution of the chromosomes.

F.—*Tripsacum dactyloides* L. Homotypic anaphase.

G.—*Tripsacum pilosum* Scribn. & Merr. Heterotypic anaphase, showing 3 lagging chromosomes.

H.—*Tripsacum lanceolatum* Rupr. Homotypic anaphase, showing many chromosomes lagging on the spindle.

I.—*Tripsacum lanceolatum* Rupr. Homotypic telaphase, showing extruded chromatin material in the cytoplasm.


A
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I

A FUSARIUM BULB ROT OF ONION AND THE RELATION OF ENVIRONMENT TO ITS DEVELOPMENT[1]

By J. C. WALKER, *Pathologist*, and E. C. TIMS, *Collaborator, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

The Fusarium bulb rot of onion was first brought to the attention of the senior writer in 1918 by its occurrence in the large onion-set-growing region in the vicinity of Chicago. It was then one of the three most serious diseases of the onion-set crop, and since that time its importance has increased rather than diminished. A similar disease had been reported from Ohio in 1910 by Selby (*8, p. 416*)[3], from Connecticut in 1915 by Clinton (*1*), and from Japan in 1914 by Hanzawa (*4*). Since 1918 the reports of Fusarium rot of onion in the field as well as in storage and transit have become more numerous and widespread, while the toll of losses due to this disease is rapidly increasing (*2, 3, 6*).

Laboratory investigations of the disease were begun by the writers in 1921 with material collected in Illinois. Shortly after that time it was learned that Dr. G. K. K. Link was initiating studies upon the disease as it occurred in various other onion-growing regions. Since he was in the better position to assemble and study strains of Fusarium on onion from widely different sources, the writers have not attempted to determine whether or not one or more species of Fusarium may cause the bulb rot. On the contrary, they have confined themselves to the study of a single strain of Fusarium which appears to be the primary cause of the bulb rot in the onion-set sections of Illinois. It is not maintained however that this is the sole cause of the disease in this or other sections, for further survey and study may show other strains or species to be pathogenic on onion bulbs.

This strain belongs to the section Elegans and resembles *Fusarium hyperoxysporum* Woll. in some respects and in others it is similar to *F. redolens* and *F. oxysporum*. The description of *Fusarium cepae* by Hanzawa, though not as complete as might be desired, coincides very closely with the organism under discussion, and the symptoms of disease are identical. Therefore the present strain is referred to this binomial and a fuller description of its characters is given.

DESCRIPTION OF THE DISEASE

Under midwestern conditions this disease appears usually some time after the 1st of July. A progressive yellowing and dying back from the tips of the leaves is the first sign. (See Pl. 1.) The rapidity of this development will vary, sometimes the aerial part dying completely within one or two weeks and in other cases the decay extending over a much longer period. Appearance of newly affected plants may continue until harvest. When the early signs of the disease appear above ground, decay has already started at the stem plate.

[1] Received for publication Feb. 20, 1924.

[2] The writers are indebted to the department of plant pathology, University of Wisconsin, for laboratory and greenhouse facilities placed at their disposal during the course of this investigation.

[3] Reference is made by number (italic) to "Literature cited," pp. 693-694.

The roots commonly turn pink and gradually decay until eventually the entire root system may disappear. A semiwatery decay affecting all the tissues of the succulent scales starts from the base upward. In early infections this decay may continue so as to almost completely destroy the bulb by harvest. In other cases incipient infections at harvest continue to advance during storage and transit, finally leaving dry, shrivelled mummies. In the case of red onions it is common for the anthocyan pigment in the outer epidermis to turn green in color for a considerable distance in advance of the actual decay, indicating a change in reaction of the cell sap. Fusarium infection of the bulb is often associated with insect wounds, and since this organism is primarily a wound parasite the coincidence of severe epiphytotics of the disease and maggot injury is common.

THE CAUSAL ORGANISM

SOURCE OF PURE CULTURE

The culture of Fusarium used in the following experiments was isolated from typically diseased specimens of White Portugal onion sets collected in storage at Morton Grove, Ill. Spore dilution plates were first made, and from a subsequent transfer a pedigreed strain from a single spore was secured. This strain was used for all of the experimental studies.

MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE ORGANISM[4]

The morphological and cultural characters of the organism as determined from cultures on various media are as follows:

Fusarium cepae (*Hanzawa*) *emend.* Microconidia usually not numerous. Macroconidia gradually attenuate toward the apex, distinctly pedicellate and uniformly curved throughout; typically 3-septate, 35 by 4 (21 to 47 by 3 to 5) microns; from small to medium sporodochia (up to 1.5 mm. in diameter), often converging into pionnotes; chlamydospores intercalary and terminal and commonly present in conidia; aerial mycelium typically scant; when present, short, white to gray; color of conidia from light brown[5] when grown in the dark, to ochraceous salmon or light ochraceous buff when grown in diffuse light; no sclerotia have been observed on any medium; color of substratum quite variable, on slightly acidified hard potato agar plates in diffuse light, from dark vinaceous purple to vinaceous purple; another series on same medium under same conditions color from chocolate to Hessian brown.

Measurements of spores on the various media are as follows:

On hard lima bean agar culture, 9 days old, conidia from pionnotes: Mostly 3-septate, range 21 to 44×3 to 5μ, average 35.6×4μ.

On hard lima bean agar, culture 20 days old, conidia from pionnotes: Mostly 3-septate, range 30 to 47×4 to 4.6μ, average 40×4.3μ.

On potato stem plug, culture 21 days old, conidia from small sporodochium: Mostly 3-septate, range 26 to 42×3 to 5μ, average 34×3.9μ.

On raspberry cane plug, culture 24 days old, conidia from small sporodochium: Mostly 3-septate, range 30 to 38×3 to 5μ, average 34.5×4μ.

On oat agar, culture 10 days old, conidia from large sporodochium: 75 per cent, 3-septate, range 33 to 44×3 to 4.5μ, average 35.7×3.7μ; 24 per cent, 4-septate, range 35 to 45×3.2 to 4.8μ, average 39.2×3.6μ.

On hard oat agar, culture 250 days old, chlamydospores (in conidia): Abundant, 0-septate, range 7.6 to 10.5×5 to 8.8μ, average, 8.2×7.9μ.

[4] The writers are indebted to Miss Helen Johann for many helpful suggestions in connection with these studies.

[5] Designations of color are made according to Ridgway (7).

On hard oat agar, culture 130 days old, chlamydospores (in conidia): 0-septate, range 6 to 10.5×5.5 to 8.9μ, average 8.3×8μ.

On onion agar, culture 275 days old, chlamydospores (in conidia): 0-septate, range 6.7 to 9.6×6 to 9.2μ, average 7.9×8.4μ.

On malt agar, culture 80 days old, chlamydospores, terminal and intercalary, very variable in size and shape from spherical to ovoid, mostly 1-celled, occasionally 2-celled; 1-celled range, 5.7 to 9×5.7 to 9μ, average 7.5×7.5μ; 2-celled, range, 7.5 to 10×12 to 13.5μ, average 8.0×12.5μ.

After comparison with the descriptions by Wollenweber (*10*) and Sherbakoff (*9*) it is evident that this form belongs to the section Elegans of the genus Fusarium and within that group lies very close to *Fusarium redolens* Woll., *F. hyperoxysporum* Woll. (*F. lutulatum* Sherb.) and *F. oxysporum* Schlecht. It differs from the first of these chiefly in the type of spore curvature. Its macroconidia are uniformly curved throughout (fig. 1), while a distinguishing character of *F. redolens*

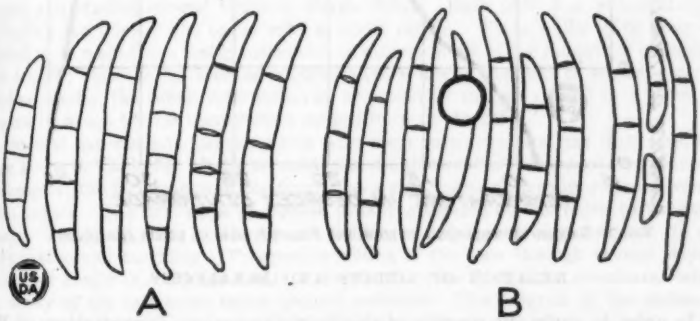

FIG. 1.—Camera lucida outlines of conidia of *Fusarium cepae* A. Sporodochial conidia from 24-day-old culture on red raspberry cane plug. B. Pionnotal conidia from 10-day-old culture on hard lima bean agar

is that the conidia are typically broader toward, more curved near, and sometimes suddenly constricted at the apex. The strain here discussed never showed this last character. It agrees closely with *F. hyperoxysporum* as to size and shape of conidia, but differs decidedly in its general appearance on various media. It differs from *F. oxysporum* in having slightly larger spores and in the total absence of sclerotia. It agrees with Hanzawa's description (*4*) of *Fusarium cepae* as to septation, curvature, and length of conidia, but differs slightly as to width of conidia and size of chlamydospores. Its measurements of 3-septate conidia average 3 to 5μ; he records 4.5 to 6.3μ. In his figures he has illustrated the swollen conidia; and if we assume that he also measured the maximum width of the swollen conidia, rather than the width at the septa, the discrepancy is probably accounted for. Wollenweber (*11, p. 173*) mentions the importance of considering this point in comparing the descriptions of Fusaria.

TEMPERATURE RELATIONS

Petri dishes of equal size containing equivalent amounts of hard potato agar (2 per cent glucose) were inoculated in the center with the organism and placed in duplicate in a series of incubators ranging from 4° to 35° C. Growth occurred over the entire range, but very slowly at the extremes. The diameter of the colonies after five days was taken as a criterion of comparison, the results being represented graphically in Figure 2. There is a gradual increase in the rate of growth up to 25°, with a distinct optimum at about 25° to 28°, and a rapid drop beyond 30° C.

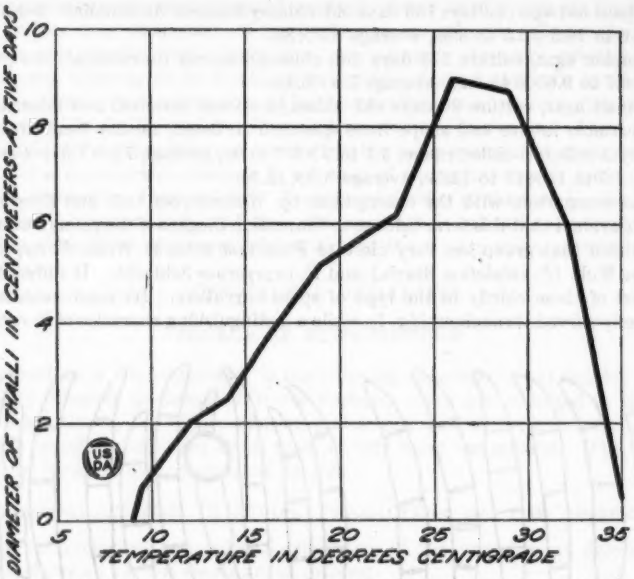

FIG. 2.—Relation of temperature to growth of *Fusarium cepae* on potato agar plates

RELATION OF ACIDITY AND ALKALINITY

In order to study the reaction of the fungus to various concentrations of H and OH ions the methods outlined by Karrer and Webb (*5*) were followed with slight modifications. Erlenmeyer flasks of 150 cc. capacity containing 50 cc. each of Richard's solution adjusted to various P_H values were inoculated with equivalent amounts of a spore suspension of the fungus. After 10 days' incubation at 22° C. the mycelial mats were filtered on weighed filter paper, dried at 90° C. and the weight determined. The P_H values of the solutions at the beginning of the experiment and after removal of the mycelium were determined. These with the dry weights of the mycelium are given in Table I.

TABLE I.—*Growth of Fusarium cepae in Richard's solution adjusted to various P_H values after 10 days at 22° C.*

P_H value		Dry weight of mycelium
At beginning of experiment	After removal of mycelial mat	
		Grams
1.8	1.8	No growth.
2.2	2.2	0.0050
2.8	2.8	.0256
2.8	2.8	.0240
5.0	4.6	.2282
6.6	6.8	.2340
7.4	7.2	.1845
8.2	8.0	.1460
8.4	8.2	.0209
9.2	8.2	No growth.
9.6	8.4	No growth.

The fungus grows best in a slightly acid medium. There was very little change in the reaction of the medium during the experiment, even at the points where most profuse growth occurred. The acidity of the scale tissue of nearly mature onion bulbs of the red, yellow, and white types was found to vary between P_H 5.3 and 5.4. The fungus grows well beyond the acidity limits ordinarily encountered in the host tissue. Moreover one would expect the reaction of the soil to materially affect the fungus only in the case of very high acidity or alkalinity.

PATHOGENICITY

The repeated isolation of this species from decaying onion bulbs was evidence of its pathogenic properties. This fact was further substantiated by inoculation of growing onion plants and of mature bulbs.

INOCULATIONS IN THE GREENHOUSE

Apparently healthy onion sets were used; they were prepared by removing the dry outer scales, sterilizing in 1 to 1,000 mercuric chlorid solution for 15 minutes, and rinsing several times in sterile water. Each bulb was wounded by piercing the base of the scales with a sterile needle. Those bulbs to be inoculated were placed in a water suspension of macroconidia of the fungus for an hour or longer; those to be used as controls were similarly exposed to sterile water. Subsequently the bulbs were removed and planted in pots of soil in a greenhouse in which the air temperature ranged from 18° to 24° C.

Several experiments following this plan were conducted and the final results are given in Table II. Both inoculated and uninoculated bulbs produced leaves promptly, the top growth of the former, as a rule, being noticeably reduced from the outset. The first signs of disease usually appeared on the tenth to twelfth day. The green color at the tips of the leaves faded somewhat followed by a gradual loss of turgidity. Progressive wilting of the tops through several days resulted finally in complete death of the plant. (See Pl. 2, A.) Simultaneously a decay of the leaf bases below ground occurred. The progress of the disease, as will be shown later, is materially influenced by temperature.

TABLE II.—*Results of greenhouse inoculation experiments with Fusarium cepae*

Experiment number	Number plants inoculated	Plants diseased	Number of controls used	Condition of controls at end of experiment
		Per cent.		
1	18	100	18	Healthy.
2	24	100	24	Do.
3	14	100	14	Do.
4	32	100	24	[a] Do.
5	10	100	8	Do.

[a] One bulb affected with bacterial decay; no evidence of Fusarium rot present.

One hundred per cent infection resulted in each inoculation experiment while the controls remained free from Fusarium rot. The fungus was repeatedly reisolated from diseased plants and was proved to be identical with the original culture. When wounded bulbs were planted in sterilized soil inoculated with a spore suspension of the organism, the disease developed in a quite similar manner and 80 per cent of the plants became infected.

It is quite evident that the fungus is an aggressive wound parasite of onion plants. In order to determine whether or not the fungus is capable of infecting through the unbroken surface, 18 apparently sound sets were planted in inoculated soil. The same number of bulbs were planted in sterilized soil as controls. In four of the plants in inoculated soil the characteristic symptoms of the disease developed and from them the fungus was reisolated. The other plants remained healthy, as did the controls. It thus appears that the fungus will invade apparently sound plants but the percentage of infection is much less than in the case of wounded plants. It is well to recall here the common observation that

this Fusarium bulb rot is associated with onion maggot injury. It is obvious from the experimental evidence just given that mechanical injury to the base of onion bulbs due to insects or other causes will greatly facilitate infection by *Fusarium cepae*.

INOCULATIONS IN THE FIELD

Inoculation experiments were continued out of doors at Madison, Wis., in 1922 on soil which had never previously grown onions. The soil was inoculated at the time of planting (April 22) by the addition of a spore suspension of the organism. Plats of uninoculated soil were planted as controls. Bulbs were grown from bottom sets of White Portugal, Red Wethersfield, and Yellow Strassburg varieties, and from seed of Crystal Wax Bermuda, Crimson Globe, Silver Globe, Golden Globe, and White Portugal varieties of onion. Plantings from seed of White Welsh onion (*Allium fistulosum*) and leek (*Allium porrum*) were included. The development of the disease followed closely that observed previously under natural field conditions. Approximately equal numbers of plants from inoculated and uninoculated plats were examined on August 8. The results are given in Table III. It is significant to note that infection took place in the case of every variety of common onion (*Allium cepae*) tried. Varieties representing each of the three color types (white, yellow, and red) were included and no marked differences in varietal susceptibility were noted. The Welsh onion and leek are apparently decidedly resistant.

TABLE III.—*Results of field inoculations with Fusarium cepae upon different varieties of common onion and upon Welsh onion and leek*

Method of propagation	Variety	Number of bulbs examined at end of season	Per cent showing Fusarium bulb rot	Condition of controls
From sets	Red Wethersfield, White Portugal, Yellow Strassburg	215	22	Healthy.
	Red Wethersfield	150	15	Do.
	Crystal Wax Bermuda	300	2	(a)
From seed	Red Globe	175	12	Healthy.
	White Globe	295	18	Do.
	Yellow Globe	75	8	Do.
	White Portugal	300	7.5	(b)
	White Welsh	400	0	Healthy.
	Leek	400	0	Do.

[a] No Fusarium rot in checks; results were obscured by the abundance of soft rot in both inoculated plants and checks following early maturity of this variety.
[b] One bulb was diseased and Fusarium isolated therefrom.

INOCULATIONS IN STORAGE

Although the fungus was found constantly associated with the decay of onion bulbs, the first attempts to reproduce the disease by artificial inoculation of mature bulbs were unsuccessful. Insertion of spores into wounds at the base of the bulbs did not result in infection under ordinary storage conditions, although the disease was known to progress in naturally infected bulbs in the same environment. Holding of the bulbs continuously at a high relative humidity was undesirable because this condition brought about almost immediate sprouting. It was found, however, that the typical bulb rot could be produced if the bulbs were inoculated as indicated above and held for 48 hours in the saturated atmosphere of moist chambers at room temperature and then placed in wire baskets under ordinary laboratory conditions. Thirty onion sets thus inoculated and incubated at 22° C. were completely decayed after 30 days while the same number of controls showed only slight desiccation and remained perfectly healthy. The experiment was successfully repeated with large bulbs of both the white and red types. In the case of the small sets the decayed tissues dried out rapidly and a "dry rot" was produced. With the larger bulbs the greater amount of succulent tissue yielded a somewhat more "watery decay" and the greater amount of moisture brought about the production of more aerial mycelium which was most pronounced as a white fluffy mass at the base of the bulb. (See Pl. 2, B.)

RELATION OF SOIL TEMPERATURE AND MOISTURE TO THE DISEASE

The importance of studying the relation of environmental conditions to the occurrence of this disease is obvious. Since the organism is a soil fungus and infection occurs normally in the subterranean parts of the plant, soil temperature and soil moisture were considered first.

Temperature studies were carried out in Wisconsin soil temperature tanks. Inoculations were made, as previously described, by first placing the wounded bulbs in a spore suspension and then planting in metal cans of soil, four bulbs to a can. The soil moisture was adjusted to 80 per cent of the water-holding capacity, and the cans were then placed in the constant temperature tanks held at 30°, 26°, 22°, 19°, 15°, and 12° C. An equal number of uninoculated controls were included in each case. The air temperature ranged from 15° to 20° and was uniform for all tanks.

No signs of the disease developed at 12° C. even after several weeks. All of the inoculated plants held at 15° to 30° eventually succumbed to the typical wilt of the tops and decay of the bulbs, but a decided difference prevailed in the progress of the disease at the various temperatures. The bulbs all sent up tops very quickly. The first signs of the wilt were noted on the tenth day at 26° and at 30° and the disease progressed very rapidly at these temperatures, resulting in entire collapse of the plants by the twentieth day. The controls at these temperatures had reached a height of eight inches at this time. The plants at 22° and at 19° were proportionately slower in succumbing to wilt. At 15° there was no sign of wilt until the twenty-fourth day and the last of the four plants was not affected until the thirty-sixth day. The experiment when repeated and extended to 32° gave confirming results and the disease was nearly as active at 32° as at 28°. (See Pl. 3.) The controls all remained healthy with the exception of one plant at 30° which wilted slightly.

The results show the fungus capable of producing the disease at a range of considerable width extending from 15° to 32° C. or above. Within these limits there is an optimum at about 28° and a gradual retardation of progress as the temperature lowers. The disease, as affecting the growing plant, is apparently inhibited at 12°.

Constant soil moistures are not so readily obtained. To avoid as much as possible variations in the can due to force of gravity upon the soil water, shallow metal pots were chosen (6 inches deep and 5 inches in diameter). Sterilized greenhouse clay loam soil was adjusted in various lots to 100 per cent, 85 per cent, 50 per cent, and 26 per cent of its water-holding capacity, as determined by the standard 10-inch cylinder. The soil was then potted, care being taken to so pack it that equivalent amounts by dry weight occupied equivalent volumes throughout the series. In the case of the highest moisture, final adjustment was made after the soil was potted. Constant moisture content was approximated by daily weighing and adjustment. At the end of the experiment moisture determinations of samples from the upper 2 inches of each of the pots were made.

In pots of soil thus prepared, bulbs wounded and inoculated in the usual manner were planted, 4 bulbs to each pot. These pots were placed in a greenhouse held at 20° to 25° C. Although the range of soil used extended from a very dry soil to one practically saturated, little difference was noted in the rate at which the disease developed. (See Table IV.) It appears that soil moisture within the range at which the onion plant will normally develop, does not materially affect the Fusarium wilt or decay after infection has once occurred.

TABLE IV.—*Relation of soil moisture to development of Fusarium cepae*

Original moisture content (percentage of dry weight)	Moisture content of surface 2 inches at end of experiment	Approximate per cent of water-holding capacity	Number of plants inoculated	Days elapsing before all plants had wilted	Number of control plants	Number of checks wilting
10.5	9.5	26	12	14	4	0
21	25	50	[a]12	20	4	0
31	33	85	12	14	4	0[b]
37	37.5	100	12	15	4	0[b]

[a] One plant had not wilted at end of experiment.
[b] One bulb rotted completely without sprouting.

The next experiment consisted essentially of a repetition of the soil temperature studies with the exception that two complete series were included—one in soil adjusted to 30 per cent and the other to 75 per cent of its water-holding capacity. The results given in Table V show again the influence of temperature on the development of the disease.

The plants in the high moisture series sprouted and grew much more rapidly than those in the lower moisture cans, and attained a much greater final growth. The same was true with the check plants in the high-temperature tanks, as compared with the lower ones, but the greatest final growth took place in the high-moisture cans in the 19° C. tank. The first evidence of the effect of the fungus on the plants appeared simultaneously in the high and low moisture cans in the 26° tank on the eighth day. A day later the inoculated plants in the 30° and 26° tanks began to wilt, followed two days later by one in the 19° tank. Although the wilt appeared in the 19° and 22° tanks shortly after it appeared at the higher temperatures, its progress was much slower in the former. This agrees with former temperature experiments. At 12° the disease did not appear in either lot after 35 days, showing this to be below the minimum temperature for its development. The results again show that there is no appreciable difference in the rapidity of Fusarium rot under widely different soil-moisture conditions. It is to be noted, however, that in all these experiments the fungus was injected into the tissue of the bulb at the beginning. Therefore the results should not be interpreted as necessarily indicating the effect of soil moisture upon initial infection but rather upon the progress of the disease after infection.

TABLE V.—*Effect of soil-moisture content upon the progress of Fusarium cepae at various soil temperatures*

Temperature	Days elapsing before first plant wilted		Days elapsing before all plants wilted		Condition of controls
	Thirty per cent water-holding capacity [c]	Seventy-five per cent water-holding capacity [d]	Thirty per cent water-holding capacity [c]	Seventy-five per cent water-holding capacity [d]	
°C.					
30	9	9	11	12	Healthy.
26	8	8	10	10	Do.
22	9	9	17	16	Do.
19	11	11	19	19	Do.
12	(a)	(a)	(a)	(a)	(b)

[a] None of the plants showed signs of wilt at 45 days.
[b] One plant attacked by Aspergillus and Penicillium but inoculated plants not affected.
[c] 11.1 per cent of the dry weight.
[d] 27.8 per cent of the dry weight.

RELATION OF TEMPERATURE TO OCCURRENCE AND DISTRIBUTION

The final interpretation of field observations in the light of these experimental data must be delayed until a thorough survey of Fusaria associated with bulb rot and a study of their morphological and physiological characters has been completed. However, it is significant to note that at least in the Middle West, where this disease is now common, it does not ordinarily appear until July or later. An examination of soil-temperature records (see fig. 3) taken at 1-inch depth in an onion field at Racine, Wis., on two extreme seasons, 1915 and 1916, shows that in this rather northerly section the daily mean does not ordinarily reach 15° C., the lower limit for this disease, for any considerable period until May 15 or later, while the optimum temperature is not reached until July 1 or later. It would therefore seem plausible to believe that soil temperature limits the progress of the disease, so far as this species of Fusarium is concerned, during the early half of the growing period.

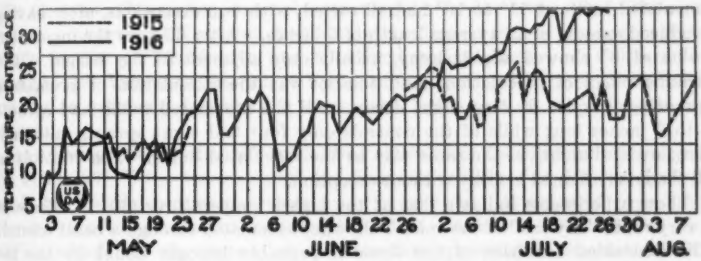

FIG. 3.—Chart from data collected at Racine, Wis., during 1915 and 1916, showing the daily mean soil temperature at a depth of 1 to 2 inches

The distribution of the Fusarium bulb rot is not sufficiently well known to interpret any limits on the basis of soil factors. It is significant, however, to note that it has appeared perhaps in its most severe form in the Walla Walla section of Washington and in the Uncompahgre section of Colorado, both of which regions are subject to very high temperatures during the last part of the onion-growing season. Moreover in a recent survey of onion diseases in Europe, the senior writer found Fusarium bulb rot doing severe damage only in the irrigated section at Valencia, Spain. Here again the disease appears in the hot midsummer and the general conditions of soil and air environment are very similar to those of the Walla Walla section. A repetition of the soil temperature experiments described above with two pathogenic strains of Fusarium collected in the Valencian section showed that these forms coincide very closely with *Fusarium cepae* in their temperature reaction.

RELATION OF STORAGE TEMPERATURE TO THE DISEASE

A thorough study of storage conditions as affecting the progress of a bulb rot should include a study of humidity as well as temperature. Since facilities for controlling humidity were not available, some preliminary experiments were conducted in which only the temperature was varied.

Experiment 1: Sixty small white onion sets were prepared for inoculation in the usual manner and divided into two equal groups. One group remained without further treatment to serve as controls while the other 30 bulbs were inoculated by insertion of macroconidia of the organism into the wound. The two groups were next placed in separate moist chambers at 22° C. for 48 hours and were

then subdivided into three equal lots as follows: Lot 1, consisting of 10 inoculated and 10 uninoculated bulbs, was placed in wire baskets in an incubator held at 21° to 22° C.; lot 2 was held at 13° to 18°; and lot 3 was held at 5 to 14°. The inoculated bulbs in lot 1, when examined 30 days later, were completely decayed, the succulent tissue having almost disappeared, leaving the outer dry scales in their original position. In lot 2 the decay was nearly as complete, desiccation not having progressed quite so far. At 5° to 14° the decay extended only through the immediate center of the bulbs. The controls remained healthy in all cases.

Experiment 2: One hundred and sixty white sets (¾ to 1½ inches in diameter) were divided into four lots of 20 inoculated and 20 uninoculated bulbs each. They were placed at 32°, 20° to 21°, 11° to 16°, and 8° C., respectively. After 19 days the inoculated bulbs at 32° were completely decayed and desiccated; at 20° to 21° the rot was well advanced but the tissue was more watery than at 32°; at 11° to 16° the inoculated bulbs were only slightly decayed but were sprouting profusely; while at 8° the decay was very slight. After 37 days the inoculated bulbs at 11° to 16° had all rotted, with two exceptions, after having produced sprouts of an average length of 5 inches. After 62 days the inoculated bulbs at 8° showed slight decay, usually only adjacent to the wound. The striking effect of inoculation upon this lot was the stimulation of premature sprouting in spite of very slight decay. All the inoculated bulbs had sprouts 2 to 6 inches long while of the controls only four had short sprouts, although they were wounded in the same way as the inoculated bulbs. The controls in all the lots remained healthy without exception.

These experiments indicate that at the higher temperatures (30° C.) there is a very rapid "dry rot" of the onion bulb when inoculated through a basal wound. The desiccated condition of the tissue is probably brought about by the increased evaporation due to high temperature and somewhat lower relative humidity. As the temperature is reduced the progress of the rot is slower until at 8° there is very slight decay but excessive sprouting. The latter is an important factor in onion storage, since sprouting makes the bulb worthless for market purposes, and thus Fusarium infection may be indirectly responsible for considerable losses on onions held at low temperatures. It is also of interest to note that the fungus is active at a lower temperature in the case of the dormant bulb than in that of the growing plant.

SUMMARY

Fusarium bulb rot of onion is a disease of increasing importance in the United States. A comprehensive survey of Fusaria associated with the disease in various parts of the world has been started by Dr. G. K. K. Link. The present paper is a study of the disease as caused by a single strain which appears to be the chief causal agent of the disease in the Middle West. A description of the disease under mid-western conditions is given.

The organism, *Fusarium cepae* Hanzawa, belongs to the section Elegans and is very close to *Fusarium hyperoxysporum* Woll., *F. redolens* Woll., and *F. oxysporum* Schlecht. Its morphological and cultural characters are recorded.

On potato agar the fungus grows over a range of from 4° to 35° C. Optimum growth takes place between 25° and 28°.

On Richard's solution the fungus grows over a range in P_H from 2.2 to 8.4; best growth occurs at P_H 6.6. The fungus thus grows well within that range of acidity or alkalinity ordinarily encountered in the host tissue or in the soil.

Inoculations were readily secured through wounds in plants growing from sets in the greenhouse. A much smaller percentage of infections was secured

when the plants were not wounded. As high as 22 per cent infection was secured in plants grown out of doors, from sets and from seed, on previously inoculated soil. Bulbs inoculated through wounds and kept under ordinary laboratory conditions very seldom became infected. Exposure in a moist chamber for 48 hours after inoculation followed by removal to laboratory conditions resulted usually in 100 per cent infection.

In controlled soil-temperature experiments the disease developed within the limits of 15° to 32° C. Most rapid development occurred at 28° to 32°. Progress was much slower as the temperature decreased. The disease did not develop at 12°.

Variation in soil moisture had little or no effect upon the progress of the disease after initial infection had taken place.

The results of soil-temperature experiments coincide with the occurrence of the disease in the field. In southeastern Wisconsin the soil temperature mean at 1 to 2 inches does not ordinarily reach 15° C. until May 15 or later while the optimum temperature for the disease is not reached until July 1 or later. The disease in this section does not appear until after the latter date. The most serious outbreaks of Fusarium bulb rot in the United States are in the Walla Walla section of Washington and the Uncompahgre section of Colorado; in Europe, the Valencian district of Spain suffers most. All three sections are subject to extremely hot weather during the latter part of the onion-growing season.

As a storage disease the bulb rot is most active at or above room temperature. At about 30° C. the tissues decay and desiccate rapidly. At 20° the decay is rapid but the tissue remains watery for a longer time. At about 15° the decay is very slow but premature sprouting is evident. At around 8° the rot is very slight indeed, but the promotion of premature sprouting in inoculated bulbs is very marked. Thus at this low temperature the indirect effect of the disease may cause heavy losses in storage or transit.

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PLATE 1

White variety of onions showing infection by the bulb-rot Fusarium as it occurs on the immature plants three or four weeks before harvest. Note the yellowing and loss of turgidity beginning at the tips of the leaves and the decay of the scales and root system starting at the stem plate. Specimens collected at South Holland, Ill., July, 1918.

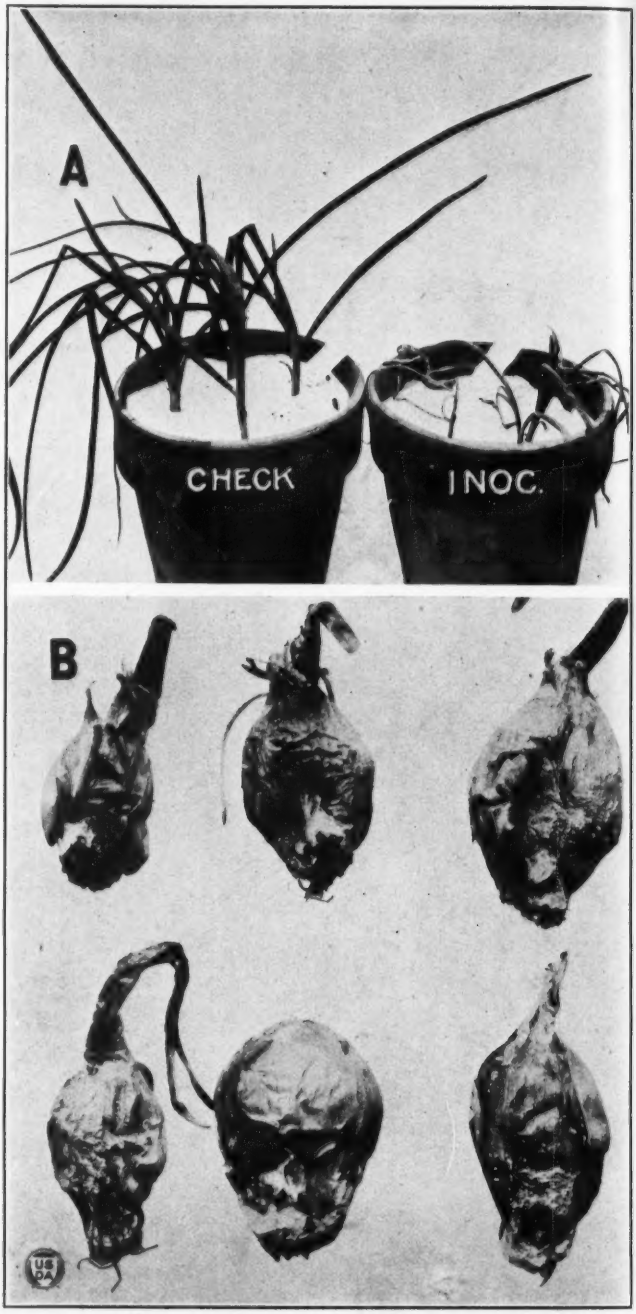
A
CHECK
INOC.
B

PLATE 2

Inoculation experiments with *Fusarium cepae*.

A.—White Portugal onion sets were inoculated with spores through needle punctures at the base of the bulbs, and planted in sterilized soil in the greenhouse at about 22° C. Checks were wounded but not inoculated. Both lots produced top growth promptly, but the inoculated plants grew less rapidly. The disease appeared as loss of turgor and yellowing at the tips of the leaves on the tenth day. Progressive yellowing and wilting continued, while the checks grew as usual. Photographed 20 days after inoculation.

B.—Large bulbs, Red Globe variety, inoculated with spores through needle punctures at base, placed in moist chambers for 48 hours, and then removed to laboratory temperature and humidity. Typical decay and shrinkage of tissue resulted with conspicuous production of mycelium on the exterior of outer rotted scales. Photographed 23 days after inoculation.

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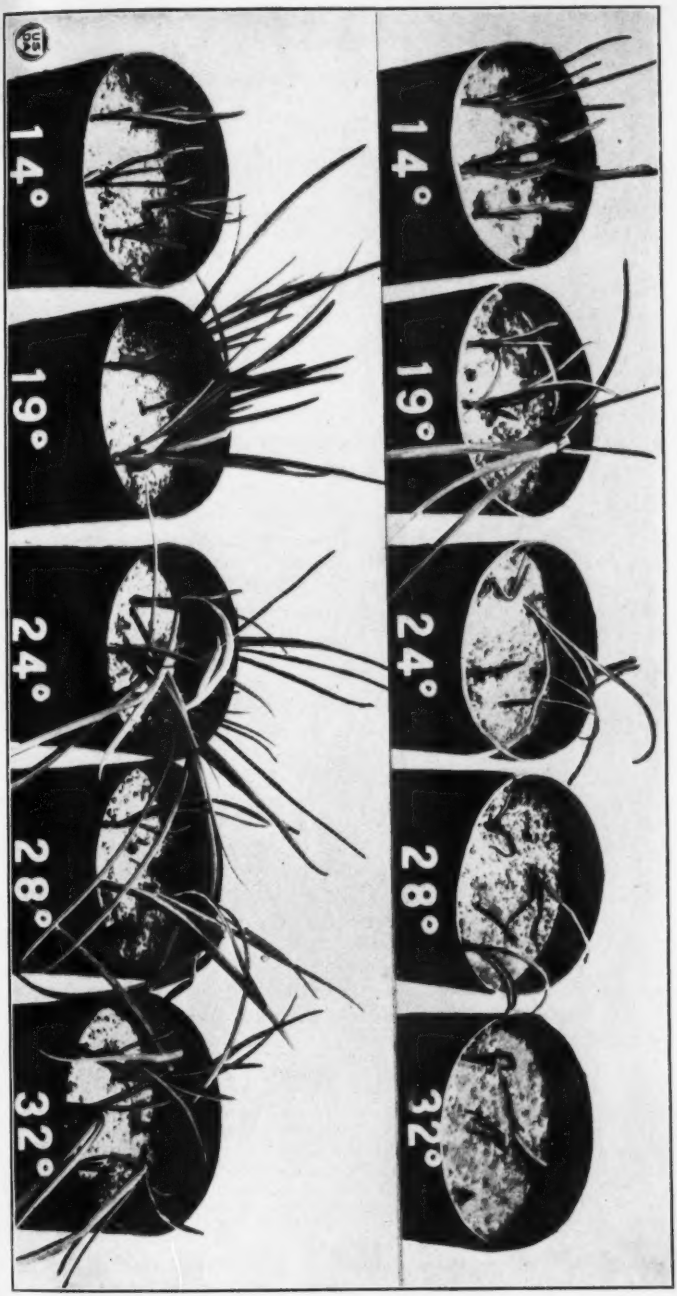
PLATE 3

Relation of soil temperature to the progress of *Fusarium cepae*.

Upper row: Plants from White Portugal sets inoculated and planted as described for Plate 2, A, and held at various soil temperatures. Lower row: Plants from uninoculated bulbs held at the same temperatures. Photographed 18 days after initiation of the experiment. The disease has not developed at 19° C. nor at 14° C. At 24° the plants have recently wilted. At 28° and 32° the plants were killed rapidly before the tops had grown to any great extent.


14°
19°
24°
28°
32°
14°
19°
24°
28°
32°

THE CHLORID CONTENT OF THE LEAF TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON[1]

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INTRODUCTION

In earlier investigations of the physico-chemical properties of the leaf tissue fluids of Pima Egyptian and Acala and Meade Upland cotton (*5*)[3] we have shown that when grown under irrigation at the United States field station in the Gila River Valley at Sacaton, Ariz., the Egyptian cotton is characterized by higher osmotic concentration, as measured in terms of freezing point depression, Δ, and higher specific electrical conductivity, κ, of its tissue fluids than either of the two Upland varieties.

The consistently higher osmotic concentration of the tissue fluids of the Egyptian cotton might be due to either greater capacity for the synthesis of organic solutes or to greater capacity for the absorption and for the retention in solution of electrolytes from the soil. The higher constants for the specific electrical conductivity, κ, of the tissue fluids of the Egyptian cotton point to the absorption and tolerance in solution of electrolytes as important factors in the differentiation of the physico-chemical properties of the tissue fluids of the two types of cotton.

In analyzing the physiological differences between the Egyptian and Upland types more minutely, therefore, it becomes of importance to determine the concentration of specific solutes in the tissue fluids.[4]

The desirability of an investigation of the chlorid content of the tissue fluids of the Egyptian and Upland types of cotton is suggested by the early observations of Kearney and Means (*7*) and of Kearney (*6*) on the growth of cotton in saline soils. Furthermore, Balls (*1*, *2*) has called attention to the relatively high chlorid content of cotton plants grown in Egypt.

In view of these observations, and of the differences in the physico-chemical properties of the tissue fluid of Pima Egyptian, Meade, and Acala Upland cotton already distinguished (*5*), the determination of the concentration of chlorids in the tissue fluids of different varieties might seem to be of particular importance in selecting crop plants which may best be grown in regions of so-called alkali soils, in many of which the content of chlorids is very high.

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[3] Reference is made by number (italic) to "Literature cited," p. 704.

[4] This has been one of the primary purposes of this phase of the work carried out on cotton by the Office of Alkali and Drought Resistant Plant Investigations, the Office of Biophysical Investigations, and the Office of Western Irrigation Agriculture, of the Bureau of Plant Industry, United States Department of Agriculture, since 1921. For the opportunity of joining in this program we are indebted to T. H. Kearney, G. N. Collins, and C. S. Scofield, in charge of the three offices mentioned.

MATERIALS AND METHODS

The data are derived from four experimental series of plants grown at the United States field station, Sacaton, Ariz. These may be numbered for convenience of reference as follows:

A.—A comparison of Pima Egyptian and Acala Upland cotton in 1920.

B.—A comparison of Pima Egyptian and Meade and Acala Upland cotton in 1922.

C.—A second comparison of Pima Egyptian and Meade Upland cotton in 1922.

D.—A comparison of Pima Egyptian and Lone Star Upland cotton in 1923.

The two or three varieties of plants to be compared were distributed uniformly over the plots available in order to avoid the influence of substratum heterogeneity (*3*, *4*). In this regard the technique was essentially that of the experiments made in 1921 (*5*).[5]

The first comparison of Pima Egyptian and Meade and Acala Upland cotton in 1922 (Series B, above) was made by planting 24 rows each 180 feet in length and consisting of six cultures of 10 feet each of Acala, Pima, and Meade.[6]

In the collection of samples, the field was in effect divided into 216 subplots (each 10 feet long and two rows wide, and planted to one of the three varieties) by combining rows 1 and 2, 3 and 4, etc. The samples of tissue were drawn as widely and as uniformly as possible from the theoretically 20[7] plants of each subplot. The three adjoining subplots of the same pair of rows serve as units in the making of comparisons. Each unit therefore comprises determinations on Pima, Acala, and Meade plants. The samples of each unit were taken simultaneously, so that external conditions which vary with time can not influence the results.

This comparison (B) involves a first and a second series of determinations, the first based on samples collected from July 25 to August 9, the second based on samples collected from the same cultures between August 28 and September 4, inclusive.

The second comparison (C) of Pima Egyptian and Meade Upland cotton made in 1922 was based on samples of tissue collected from individual plants of the two varieties. These plants were distributed uniformly over a plot 26.5 by 400 feet in area. They were interspersed among plantings of five newly imported Egyptian varieties which do not require consideration here. The Pima and Meade plants were separated by only three hills of F_2 hybrid plants. Tissue samples were collected from August 21 to August 27, inclusive.

The comparison of Pima Egyptian and Lone Star Upland cotton in 1923 was made on an irrigation plot of the standard dimension, which was selected because of the high salt content of the soil at the north end. The south half of the plot had lain in alfalfa from 1920 to 1923. The north half was too saline to permit a growth of alfalfa, and but few cotton plants survived on this portion of the plot. The collections made from the plants of the 10-foot subrows at the south and the north half of the plot will, therefore, be discussed separately.

[5] This statement applies to the cultures made in 1922 and 1923. The few determinations made in 1920 were secured incidental to other determinations.

[6] The 24 rows were distributed over the north half of three irrigation plots, each 26.5 by 400 feet in area. Ten feet at each end were used for buffer plants in order that the end plots used might have the same cultural conditions as those in the center of the field.

[7] Because of the cold spring, germination was poor and many plants were wanting. The number was still further reduced by the development of root rot late in the season.

Samples of tissue were collected and the fluids extracted, and a number of physico-chemical constants determined [8] in essentially the manner described in an earlier paper (*5*). The chlorid content was determined by precipitating the chlorids as silver chlorid by the addition of a known amount of standard silver nitrate solution and modifying all organic substances by digesting on a sand bath with boiling nitric acid. The excess silver nitrate was then titrated with potassium thiocyanate, with iron alum as indicator.

The samples for the series of 1920 and 1922 were preserved in sealed tubes and analyzed later. In all these the precipitated silver chlorid was removed by filtration. The analyses for 1923 were made on the fresh tissue fluids. In these determinations titration was made in the presence of the precipitated silver chlorid, as has been shown to be allowable (*8*) in this method.

All results are expressed as chlorids in terms of grams of Cl per liter of tissue fluids. Duplicate analyses were made in a rather large proportion of the cases. The two determinations have been averaged to obtain the constant to be used to represent the plants unless there was some evidence that one of the two analyses was more accurate than the other. When one sample contained 10 cc. whereas the other contained less than 5 cc. the constant determined from the larger sample has generally been taken.[9]

PRESENTATION OF RESULTS

Only one pair of determinations of chlorid content are available for cotton grown in 1920. These seem to indicate a higher concentration of Cl in the Egyptian than in the Upland types.

TABLE I.—*Chlorid content of the leaf tissue fluids of Egyptian and Upland cotton grown in 1920*

Variety of cotton	Number of sample [a]	Gm. of chlorids (Cl) per liter
Pima Egyptian	(1)	6.29
Acala Upland	(2)	4.43
	(1)−(2)	+1.86

[a] The sample numbers are the same as those used to designate the determinations given elsewhere (*5*).

In each experiment except that for 1920, relatively large numbers of analyses were made on samples so taken that each determination based on tissues collected from Egyptian plants was provided with a control sample taken from Upland plants grown under as nearly as possible the same conditions.

The comparisons for these large series may be most crucially made by means of statistical constants and their probable errors. The differences between the varieties compared are, however, sufficiently large that they may be brought out by means of a mere tabulation of the analyses.

[8] These will be considered elsewhere in relation to another group of problems.

[9] We have to thank Supt. C. J. King for many courtesies which facilitated our work at Sacaton. George J. Harrison was responsible for the making of the rather complicated plantings and for the superintendence of the cultures throughout the season. We are greatly indebted to G. O. Burr, Charles W. Crane, R. D. Evans, A. H. Johnson, W. B. Sinclair, and A. T. Valentine, field assistants in 1922 and 1923, for their large part in the collection and preparation of the samples for analysis.

The data for the larger series of determinations on Pima Egyptian and Meade and Acala Upland cotton made in 1922 (comparison B, above) and for theser ies of determinations made on Pima Egyptian and Lone Star Upland cotton in 1923 [10] (comparison D, above) appear in Table II. For convenience of tabulation the chlorid contents may be grouped in classes of 0.25 gm. of Cl per liter range.

TABLE II.—*Frequency distribution of chlorid content (in terms of grams of Cl per liter of fluid) in leaf tissue fluids of Egyptian and Upland cotton as grown at Sacaton, Ariz., in 1922 and 1923*

Grams of chlorid (in terms of Cl) per liter		Cultures in 1922						Cultures in 1923					
		First series, July 25 to Aug. 9			Second series, Aug. 28 to Sept. 4			First series, July 29 to Aug. 14		Second series, Part I, Aug. 18 to Aug. 31		Second series, Part II, Aug. 24 to Aug. 30	
Class range	Class center	Pima	Acala	Meade	Pima	Acala	Meade	Pima	Lone star	Pima	Lone star	Pima	Lone star
0.126–0.375	0.250								1				
0.376–0.625	0.500								1		2		
0.626–0.875	0.750								18		15		1
0.876–1.125	1.000								23		21		1
1.126–1.375	1.250								24		16		2
1.376–1.625	1.500			1			2	2	2		12		2
1.626–1.875	1.750						3	7	1	2	1		1
1.876–2.125	2.000			1			6	11			1		
2.126–2.375	2.250			5		2	6	16	1	1	1	1	2
2.376–2.625	2.500			10			12	10		9	1	2	1
2.626–2.875	2.750		4	12	1	10	10	12		4			2
2.876–3.125	3.000		5	7		11	7	6		10			
3.126–3.375	3.250	1	13	8		12	9	2		14		2	1
3.376–3.625	3.500	3	16	11	1	13	8	3		12			
3.626–3.875	3.750	5	11	2	2	7	3	1		9		2	
3.876–4.125	4.000	5	8	6	2	1	3			4		2	
4.126–4.375	4.250	12	6	2	3	7				3		2	
4.376–4.625	4.500	5	3	3	9	5							
4.626–4.875	4.750	11	3		5	1				3		1	
4.876–5.125	5.000	8	1		6	1						1	
5.126–5.375	5.250	3			8								
5.376–5.625	5.500	4	1	1	12								
5.626–5.875	5.750	4			7								
5.876–6.125	6.000	2			5							1	
6.126–6.375	6.250	4			2								
6.376–6.625	6.500	1			2								
6.626–6.875	6.750				3								
6.876–7.125	7.000				1								
Totals		68	71	69	69	70	69	70	71	71	70	14	13

[10] The first series of determinations (July 29 to Aug. 14) for the culture of Pima Egyptian Lone Star and Upland cotton made in 1923 was drawn from the south half of the plot only. This was done because the few plants which developed in the north half were so small at this time that it was evident that two collections of leaves could not be made. It seemed desirable to allow them to stand until the second collection of samples was made. The second series of determinations (Aug. 18 to Aug. 31) is divided into two parts. Part I represents collections from the south half of the plot. It is exactly comparable with the first series. Part II comprises the few determinations which it was possible to make (Aug. 24 to Aug. 30) on the scattering plants in the north half of this plot. They will not be discussed in this place farther than to note that the distribution of chlorid content in the two types of cotton is in excellent agreement with that found in the main part (Part I) of the experiment.

TABLE III.—*Frequency distribution of chlorid content (in terms of grams of Cl per liter of fluid) in leaf tissue fluids of Egyptian and Upland cotton as grown at Sacaton, Ariz., in 1923*

Grams of chlorid (in terms of Cl) per liter		Frequencies for samples taken Aug. 21 to Aug. 27		Grams of chlorid (in terms of Cl) per liter		Frequencies for samples taken Aug. 21 to Aug. 27	
Class range.	Class center	Pima	Meade	Class range.	Class center	Pima	Meade
2.626—2.975	2.80	----------	4	7.526—7.875	7.70	9	1
2.976—3.325	3.15	----------	7	7.876—8.225	8.05	4	----------
3.326—3.675	3.50	----------	7	8.226—8.575	8.40	7	----------
3.676—4.025	3.85	----------	9	8.576—8.925	8.75	1	----------
4.026—4.375	4.20	----------	3	8.926—9.275	9.10	2	----------
4.376—4.725	4.55	----------	2	9.276—9.625	9.45	1	----------
4.726—5.075	4.90	1	3	9.626—9.975	9.80	2	----------
5.076—5.425	5.25	2	5	9.976—10.325	10.15	3	----------
5.426—5.775	5.60	1	4	10.326—10.675	10.50	----------	----------
5.776—6.125	5.95	1	2	10.676—11.025	10.85	----------	----------
6.126—6.475	6.30	----------	2	11.026—11.378	11.20	1	----------
6.476—6.825	6.65	3	----------				
6.826—7.175	7.00	7	----------	Totals	----------	48	49
7.176—7.525	7.35	3	----------				

Because of a higher and more variable chlorid content in the smaller comparison between Pima and Meade Upland cotton made in 1922 (comparison C, above) a wider class value must be used for tabulation. A class range of 0.35 gm. of Cl per liter of fluid was therefore adopted. (See Table III.)

The range of chlorid content in each class and the central values to be used for purposes of calculation are indicated in the first two columns (Tables II and III.) The frequencies show the numbers of analyses falling in each of the classes in the comparisons of Pima Egyptian and Meade and Acala Upland cotton grown in 1922 and in the comparison of Pima Egyptian and Lone Star Upland cotton in 1923.[11]

While these distributions show clearly that the chlorid content of the Upland varieties (Acala, Meade, and Lone Star) is lower than that of the Egyptian variety (Pima) with which they are compared, the chlorid contents vary considerably from sample to sample. The frequency distributions are transgressive, many of the samples of Upland tissue fluids of higher chlorid content exceeding the samples of lower chlorid content in the Egyptian cotton in the concentration of this ion. It might, therefore, be assumed that the difference between the analyses is after all a statistical one, holding in the long run but not demonstrable in individual comparisons. An examination of the analyses for the associated plants of the two types of cotton shows that this is not the case.

The relationship between the individual determinations may be best brought out by means of arrangement in correlation tables. Such tables have been prepared for each of the series, but because of their essential agreement only one (Table IV) will be published.

[11] The frequencies given in these tables represent all the available determinations for each of the varieties. In some cases determinations for one or more of the varieties of a subplot could not be made; hence the totals of these distributions differ among themselves and from those given in the table of differences below (Table V) in which only determinations based on plants which occurred in actual association are given.

The comparison between the Pima Egyptian and Meade Upland cotton as grown in a second series of cultures in 1922 (comparison C, above) appears in Table IV. Because of the higher chlorid content and the greater variability [11] of chlorid content in this experiment the tabulation has been made in units of 0.35 gm. per liter range.

In this arrangement of the data the chlorid content of Upland cultures associated with Pima plants of any chlorid content are seen to be to some extent variable. It will also be noted that there is a tendency for the chlorid content of Upland plants to shift upwards in passing from the lower to the higher classes of chlorid content in the Egyptian (Pima) cotton.

TABLE IV.—*Comparison between the chlorid contents of Pima Egyptian and Meade Upland cotton as grown at Sacaton, Ariz., 1922*

Chlorid in Meade	Totals	1	1	1	1	—	3	7	3	9	4	7	1	2	1	2	3	—	—	1	47
11.20																				1	
10.85																			1		
10.50																		1			
10.15																	1				
9.80																1					
9.45															1						
9.10														1							
8.75													1								
8.40												1									
8.05											1										
7.70	1									1											1
7.35									1												
7.00								1													
6.65							1														
6.30	2							2													2
5.95	2											1			1						2
5.60	4				1								1				3				4
5.25	4			1					1	1				1						1	4
4.90	3		1								1			1		1					3
4.55	2	1								1		1									3
4.20	3									1	1	1									8
3.85	8						1		2	2	1	1				1					7
3.50	7	1						2		2		2									7
3.15	7			1				3		2		1									4
2.80	4		1		1		2														
	47	4.90	5.25	5.60	5.95	6.30	6.65	7.00	7.35	7.70	8.05	8.40	8.75	9.10	9.45	9.80	10.15	10.50	10.85	11.20	

Chlorid in Pima

[11] Possibly the greater variability of chlorid content in this experiment is due to the facts (*a*) that the chlorid content is on the average higher in this culture, and that (*b*) the analyses are based on samples of tissue collected from individual plants instead of a considerable number of plants.

Leaving this point for the moment and considering merely the relative amounts of chlorids in the two types of cotton, we may note that if the chlorid content of the two varieties were sensibly the same, the frequencies corresponding to given values of chlorid content in the two forms under consideration should be distributed at random about the "diagonal cell"—i. e., the line of cells corresponding to identical values on the scales of chlorid content for the Egyptian and the Upland varieties under comparison, of these tables. Thus the heavy bars mark off the chlorid content which the Upland cottons would have had if they had been as rich in chlorids as the Egyptian form.

The fact that without exception in the illustrative table, and almost without exception in the unpublished tables for the other series the chlorid content of the Upland forms lies below the diagonal cell which indicates equal chlorid content in the two forms, shows how conspicuously and definitely the Egyptian and Upland types are differentiated with respect to their content of this anion.

From such a table of double entry the differences in the chlorid contents of the associated Egyptian and Upland plants may be readily determined. This has been done for the comparison between Pima and Meade and Pima and Acala cultures in 1922 and for Pima and Lone Star cultures grown in 1923. The results are set forth in Table V. These entries show that practically without exception the individual comparisons show a higher chlorid content in the Egyptian than in the Upland cotton plants.

TABLE V.—*Differences in chlorid content (grams per liter in 0.25 gm. class) of leaf tissue fluids of associated Egyptian and Upland cotton plants*

Difference in chlorid content (Egyptian less Upland)	Pima Egyptian and Meade Upland		Pima Egyptian and Acala Upland		Pima Egyptian and Lone Star Upland		Difference in chlorid content (Egyptian less Upland)	Pima Egyptian and Meade Upland		Pima Egyptian and Acala Upland		Pima Egyptian and Lone Star Upland	
	First series	Second series	First series	Second series	First series	Second series		First series	Second series	First series	Second series	First series	Second series
−0.50	------	------	------	1	------	------	+2.25	4	12	1	15	3	9
−0.25	------	------	------	------	------	------	+2.50	1	9	1	4	3	11
0.00	1	------	------	1	------	------	+2.75	------	7	------	2	------	6
+0.25	1	------	8	------	------	------	+3.00	1	5	------	------	------	3
+0.50	4	------	9	2	3	------	+3.25	------	4	------	2	------	4
+0.75	4	------	14	------	6	1	+3.50	------	7	------	------	------	1
+1.00	11	1	9	3	15	2	+3.75	------	------	------	------	------	1
+1.25	10	2	12	6	7	3	+4.00	------	1	------	------	------	------
+1.50	13	2	4	15	19	9							
+1.75	8	7	5	8	8	9		67	67	69	68	68	68
+2.00	11	10	6	9	4	9							

As noted above, there is an evident tendency for the variable chlorid content of the Upland plants to be larger when associated with Egyptian plants of a higher class of chlorid content. This is more strikingly evident in some of the unpublished tables than in the illustrative one given (Table IV). This may be most readily explained as due to both Egyptian and Upland plants being influenced in the same manner by external conditions, presumably in this case by variation of chlorid concentration in the soil. Thus we have a further illustration of substratum heterogeneity as a factor influencing the behavior of plants in field plots (*3*, *4*).

TABLE VI.—*Correlation between the chlorid content of associated plants or groups of plants of Egyptian and Upland cotton as grown at Sacaton, Ariz., in 1922–23*

	Correlation for first series		Correlation for second series.	
	Correlation coefficient and probable error $r \pm E_r$	Ratio of correlation to probable error r/E_r	Correlation coefficient and probable error $r \pm E_r$	Ratio of correlation to probable error r/E_r
First comparison of Pima Egyptian and Meade and Acala Upland cotton, 1922 (B):				
Correlation between Pima and Meade	0.6702±0.0454	14.76	0.6029±0.0534	11.8
Correlation between Pima and Acala	.6844± .0432	15.85	.6030± .0520	11.8
Second comparison of Pima Egyptian and Meade Upland cotton, 1922 (C): Correlation between Pima and Meade	.5144± .0723	7.11		
Comparison of Pima Egyptian and Lone Star Upland cotton, 1923 (D): Correlation between Pima and Lone Star	.3372± .0725	4.65	.1696± .0794	2.14

The coefficients of correlation, r, measuring the relationship between the chlorid content of associated plants or subrows of plants, are given with their probable errors in Table VI.

The coefficients for the cultures of 1922 are of about the same order of magnitude as those already published for osmotic concentration and specific electrical conductivity in cultures of Pima Egyptian, Meade and Acala Upland and hybrid cotton grown at Sacaton in 1921 (*5*). The constants for Pima Egyptian and Lone Star Upland cotton studied in 1923 are much lower. This is presumably due to the greater uniformity of the half of the plot upon which this culture was grown.

The fact that the chlorid content of associated plants is correlated is not a matter of merely physiological interest. It must be considered in determining the statistical significance of the differences between the varieties. The fact that the variability of the constants is due largely to the heterogeneity of the substratum makes it essential that the constants for any two varieties compared be based on plants which have grown in the closest association practicable, and that the constants compared comprise only individuals of the same pair.

Since the two types to be compared were grown in close association in a large number of subplots distributed over the cultural area, both were subjected to the influence of similar soil conditions. This tends to reduce the probable errors of the difference between the mean chlorid content of the Egyptian and Upland types as based on the whole series of determinations available for each culture and series of samples. These probable errors may be calculated from the standard deviation of the difference between associated determinations as obtained from the formula

$$\sigma^2_{(p-u)} = \sigma^2_p + \sigma^2_u - 2r_{pu}\sigma_p\sigma_u,$$

or by actually determining the individual differences between the Pima and Upland determinations of the associated plants and calculating the standard deviations of these differences directly.

Table VII presents the mean chlorid content in terms of grams of Cl per liter of tissue fluids for the Egyptian and Upland varieties compared in the various cultures. N denotes the number of pairs of determinations. The probable errors of the means of the individual series have been calculated by the usual formula. The standard deviations are not published since variability of chlorid

content is not discussed in this place. The probable errors of the differences have been calculated with due regard to the correlation between associated plants by the formula given above.

In all comparisons the average chlorid content of the Egyptian cotton is higher than that of the associated Upland plants. The differences are many times as large as their probable errors and are unquestionably significant.

TABLE VII.—*Mean chlorid content (in terms of grams of Cl per liter) of tissue fluids of Egyptian and Upland cotton as grown at Sacaton, Ariz., in 1922–23*

	N			Difference between Egyptian and Upland cotton		
	Number of analyses	Mean chlorid content for Egyptian cotton	Mean chlorid content for Upland cotton	Absolute difference and probable error	Ratio of absolute difference to probable error	Percentage difference
First comparison of Pima Egyptian and Meade and Acala Upland cotton, 1922 (B):						
First series, July 25 to Aug. 9—						
Comparison of Pima and Meade	67	4.7388±0.0642	3.1082±0.0538	1.6306±0.0488	33.4	52.5
Comparison of Pima and Acala	69	4.7355±.0626	3.6848±.0446	1.0507±.0457	23.0	28.5
Second series, Aug. 28 to Sept. 4—						
Comparison of Pima and Meade	67	5.2276±.0676	2.7799±.0514	2.4478±.0550	44.5	88.1
Comparison of Pima and Acala	68	5.2352±.0647	3.4522±.0501	1.7831±.0528	33.8	51.7
Second comparison of Pima Egyptian and Meade Upland, 1922 (C): First series, Aug. 21 to Aug. 27—Comparison of Pima and Meade	47	7.8862±.1266	4.2670±.1120	3.6191±.1183	30.6	84.8
Comparison of Pima Egyptian and Lone Star Upland cotton, 1923 (C):						
First series, July 29 to Aug. 14—Comparison of Pima and Lone Star	68	2.4412±.0406	1.0515±.0239	1.3897±.0396	35.1	132.2
Second series, Aug. 18 to Aug. 31—Comparison of Pima and Lone Star	68	3.3088±.0477	1.1434±.0302	2.1654±.0520	41.7	189.4

When the differences are expressed in percentages of the chlorid content of the Upland type instead of in terms of grams of chlorid (as Cl) per liter of tissue fluid, the true significance of the differentiation with respect to chlorid content becomes fully apparent. The Egyptian cotton is from 28.5 to 189.4 per cent richer in chlorids than the Upland cotton grown as a control.

The causes of these variations in the differences from culture to culture must be a subject for further investigation. In passing we may note that in the comparison involving Pima Egyptian and both Meade and Acala Upland cottons the difference between Pima and Meade is greater than that between Pima and Acala in both the first and second series of determinations. We may also point to the fact that in both cultures in which two series of determinations were made the differentiation of the Egyptian and the Upland types is greater in the series of analyses made later in the season. Both of these problems will receive more detailed consideration later.

SUMMARY

This paper presents the results of a detailed comparison of the chlorid content of the leaf tissue fluids of Egyptian and Upland cotton.

Several hundred determinations based on an American Egyptian variety, Pima, grown in comparison with one or more of the Upland varieties, Acala, Meade, and Lone Star, show that almost without exception the chlorid content is higher in the tissue fluids of the Egyptian than in those of Upland cottons.

The means for seven series of determinations show the chlorid content of the Egyptian type to be from 28 to 189 per cent higher than that of the Upland type.

This is clearly one of the factors in determining the higher osmotic concentration and specific electrical conductivity demonstrated in an earlier paper (*5*) on the tissue fluids of two of these varieties.

The higher chlorid content may indicate a greater capacity of the Egyptian type for growth on saline land.

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THE RESISTANCE OF OAT VARIETIES TO STEM RUST [1]

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INTRODUCTION

An essential preliminary to breeding for rust resistance is a knowledge of the resistant varieties already in existence. An additional requirement, in the case of cereal rusts at least, is a knowledge of the physiologic specialization within the species of rust under consideration.

Since the discovery by Stakman and Piemeisel (*20*) [2], in 1917, of a second race of wheat stem rust, differing in its range of power of infection from the rust which they had been using, our knowledge of physiological races in cereal rusts has developed rapidly. In 1918 Levine and Stakman (*8*) reported a third race, and Melchers and Parker (*11*) still another. In 1919 Stakman, Levine, and Leach (*19*) distinguished about 12 physiological races and, in 1922, Stakman and Levine (*16*) reported a total of 37. Miss Newton (*13*), in 1922, made a survey of wheat stem rust in Canada and found there 14 of the 37 physiological races which are known to occur in the United States.

The discovery of physiologically distinct races of wheat stem rust led to similar studies of other cereal rusts. Hoerner (*7*), in 1919, working on *Puccinia coronata* Cda., tested many specimens of rust on certain varieties of oats chosen as differential hosts, and concluded that probably there are four physiological races of crown rust of oats. Melhus, Dietz, and Willey (*12*), in 1922, tested a wide range of grass hosts with crown rust from cultivated oats and wild grasses and distinguished four physiological races. They state (*12, p. 234*) that "*Avena sativa* was a common host with varying degrees of infection for all the forms of crown rust studied."

In 1923 Mains and Jackson (*10*), in a preliminary report of their work on leaf-rust of wheat, *Puccinia triticina* Erikss., state that this rust consists of at least 12 races. Levine and Stakman (*9*), 1923, find "at least two and probably three distinct biologic forms of *Puccinia graminis secalis*."

Stakman, Levine, and Bailey (*17, 18*), in a preliminary report and a later report on oat stem rust, *Puccinia graminis avenae* Erikss. and Henn., state that "four, and perhaps five, biologic forms of this rust can be identified by their action on Victory (C. I. 1145), White Russian (C. I. 1614), and Monarch Selection."

Parker (*15*) has published a comprehensive review of the literature on the rusts of oats.

A few extensive tests have been made in the search for varieties of oats that are resistant to stem rust. Vavilov (*21, 22*), working in Russia, inoculated 450 varieties of wild and cultivated oats with stem rust and found but two that were not fully susceptible. *Avena sativa* var. *brunnea* Kcke. was graded 2½ (using a scale in which 0 represents immunity and 4 complete susceptibility) and *Avena*

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[2] Reference is made by number (italic) to "Literature cited," pp. 718–719.

diffusa var. *montana* Al. was graded 3. The rest were uniformly 4. These two are low, slender-strawed varieties of small value. Vavilov pointed out that black rust of oats is a weakly specialized fungus which lives freely on several genera besides Avena, and states that "we come to a simple statistical conclusion as to the very slight probability of plant breeders finding oats resistant to black rust."

Parker (*14*) tested 120 strains of oats with both crown and stem rusts. Only White Tartarian and Ruakura Rustproof showed pronounced resistance to stem rust. Later, Durrell and Parker (*1*) published the results of a five-year test of about 200 lots of oats. The following were found resistant to stem rust: White Russian (White Tartar), Ruakura, Green Russian, *A. sativa grisea*, *A. orientalis mutica*, and *A. barbata*.

A beginning has been made in the breeding of oats for resistance to stem rust. Garber (*4*, *5*) made crosses between White Russian (White Tartar), a rust-resistant oat with side panicle, and Minota and Victory, susceptible forms with open panicle. He found that "rust resistance is inherited as a dominant character depending on a single factor difference for its expression," and that rust reaction and panicle type are independent in their inheritance. Griffee (*6*) reports the rust test of the F_3 generation of these crosses. Of the resistant F_2 families, one-third bred true for resistance in the F_3 and two-thirds segregated.

TABLE I.—*Result of inoculation of oat varieties with stem rust at Berkeley, Calif., in the summer of 1920, as measured on a scale of 0 to 4, in which 0 represents immunity and 4 represents complete susceptibility* [a]

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
Avena brevis Roth.						
var.	R 134d		4	3+	2+	3−
Do.	R 142a		4−	3+	3−	4−
Do.	R 289a	1783	3	3	3+	3+
Avena fatua L.						
var.	R 290a	1779	4	4	3+	4−
var. California Wild Oat. (Lemmas slightly 2-toothed)	C 1024		4−	3+	3+	4
Avena nuda L.						
var. *chinensis*	R 301		4−	4−	3	4−
var. Chinese Hull-less	W 686	298	4−		3	
var. *inermis*	R 294a	1768	3+	4	3	3+
var. Liberty	R 292a	845	4−	3+	3	4−
Do.	R 293a	1769	3	4−		3+
Do. (Differs in having geniculate awns)	C 1000			3		4
Avena sativa L.						
var. Abundance	R 289a	731	4−		4−	3
var. Abyssinian	R 308a	1747	4	4	4−	3
var. Albion	R 270a	729	4−	3	3	3
Do.	R 304a	729	4−	4	3	4−
Do. (Iowa 103)	I 51	729		4−		3−
var. *aristata*	R 35g		3	3+	3−	4−
var. *aurea*	R 7s		4−	3+		4−
Do.	R 328a	1772	4−	4	3	4−
var. Awnless Probsteier	R 114d	1888	4−	3	3+	4
var. Banner	R 271a	160	4−	3+	4	4
Do.	C 1031		3+	3−	3+	4−
Do.	W 764	1191	4	3+	4	4−
var. Belyak	R 115e	1899	4−	3	3+	3+
Do.	R 306a	1725	4	4	4−	4

[a] In the tables, the lot numbers indicating the different selections are preceded by various capital letters, which indicate the sources from which the seed was received. The lot numbers following the letter "C" are accession numbers of the California Agricultural Experiment Station. The lots designated by the letter "I" were received from the Iowa Agricultural Experiment Station and the numbers are nursery row numbers or selection numbers of the Department of Botany and Plant Pathology. The one lot bearing the letter "K" was received from the Kansas Agricultural Experiment Station. The numerous lots bearing the letter "R" were received from Dr. G. M. Reed, then pathologist in charge of smut investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the numbers are those used by him in the oat-smut nursery. The lots following the letter "W" were received from the Washington Agricultural Experiment Station, and the numbers are accession numbers of that station.

TABLE I.—*Result of inoculation of oat varieties with stem rust at Berkeley, Calif., in the summer of 1920, as measured on a scale of 0 to 4, in which 0 represents immunity and 4 represents complete susceptibility*—Continued

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
Avena sativa L.—Continued						
var. Big Four	R 272a	179	4	4	4–	4
Do	R 307a	1641	4–	4	3+	4–
Do	W 1036		3	3+	4	4–
var. Black. (Received as *Avena sterilis*)	R 295a	691	3–	3+	3	4–
var. Black American	R 185a	549	4	3	3+	4–
var. Black Diamond	R 116e	1878	3	3	3+	3
var. Black Mesdag	R 117e	1877	4–	4–	3	3
var. *brunnea*	R 81		4–	3+	3	4–
var. Canadian	R 119e	1892	3+	4–	4	3+
Do. (Barley Oat)	W 1179	1806	4–	3+	4	3–
var. Caucasus	R 279a	1754	3+	4–	4	4–
var. C. I. 602 (of Etheridge)	R 145a	602	4–	3–	3	3+
var. C. I. 603 (of Etheridge)	R 146a	603	4	3–	3–	4
var. Culberson. (The three lots differ morphologically)	R 192a	273	4	4–	4	3
Do	R 193	273–V		4–	4	4–
Do. (Listed as Culberson Winter)	C 1022		3+	4	4–	3–
var. Danish	C 1032		3–	2+	4	4–
var. Danish Island. (Awns vary in the 4 lots)	R 149a		4	3+	4–	4
Do	R 309a	1669	4–	4	4	4–
Do	R 310a	1666	4–	4	4–	4–
Do	R 311a	1684	4–	4	3	3
var. Dryland	W 767	1193	3–	3–	4	3+
var. Dun	C 1025		3–	4	4–	3+
var. Dwarf Culberson	R 193a	748	4–	3	4–	3
var. Early Champion	R 197a	1623	4	4–	3+	3+
var. Early Gothland	R 162a		4	3+	3+	4–
var. Early Mountain No. 2. (Probably Golden Drop)	R 273a	656	4–	4–	3+	4–
var. Garton 473. (Received as Garton's Regenerated Yielder)	C 1033		3+	2+	4	3+
Do. (Received as *Avena sativa orientalis*)	R 297a	1613	3+	4–	4–	3
var. Golden	R 313a	1750	3+	4	4–	3+
var. Golden Beauty	R 314a	1748	4	4	4–	3+
var. Golden Fleece	R 315a	1749	3+	4	4	4–
var. Golden Rain. (Probably Golden Drop)	R 275a	493	4–	4–	3+	3
var. Golden Rustproof	R 316a	1751	4	4	4–	3
Do	W 1055	1929	3+	4–	4	3+
var. Gray. (Probably a spreading-panicled segregate from Garton Gray)	W 663		4–	3	4	4
var. Green Russian	R 121e		3+	3	3	4
Do. (Contains a resistant strain)	R 203a	1683	3+ & 1–	3+ & 1–	3–	1– & 4
Do. (Contains a resistant strain)	R 276a	827	4	4– & 1	3+	1– & 3+
Do	I 91–4			1–		2–
var. Guyra. (Received as Gluysa)	C 1019	2034	4–	4	3–	3
var. Hatchett. (Received as Black Oat)	R 296a	838	3	3+	4	3+
var. Hay	R 317a	1622	3+	4–	2+	3
var. Heavyweight Champion	R 318a	1690	3	4	3+	4
var. Highland	W 772		2	2+	3	4–
var. Joanette. (May be X type of infection)	R 125e	1880	3 & 0	3 & 1–	1 to 2+	1 to 2+
var. June	R 157a	1902	4	3	3–	4
var. Kherson	C 1021		3+	4	2	3–
Do	W 1047	1198	3+	3	2+	4–
Do. (Wisconsin Pedigree No. 156)	C 1029		4–	4–	4–	3–
var. Kherson Selection (of Etheridge)	R 159a	1905	4	3	2–	3+
var. *Krausei*	R 329a	1771	3+	4	4	4–
var. Lincoln	C 1015		3+		3+	4–
var. Long White	C 1018		3–	4	4–	4–
var. Maine No. 340	W 1197	1935	3+	4	3+	3
var. Minnesota No. 26	R 278a	721	3+	4–	3+	4
var. Monarch Selection (of Etheridge). (Received as Monarch)	R 161a	1879	4	3+	4	4–
var. Montana	R 285a	1761	3	3+	4–	4
var. Mortgage Lifter	W 775		3	3	3+	3+
var. *nudica*. (Awns vary in the different lots)	R 42k		4–	3	3+	4
Do	R 43c		3+	3+	4–	4–
Do	R 49e		3–	2+	4–	4
Do	R 51g		3	3	3–	3+

TABLE I.—*Result of inoculation of oat varieties with stem rust at Berkeley, Calif., in the summer of 1920, as measured on a scale of 0 to 4, in which 0 represents immunity and 4 represents complete susceptibility*—Continued

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
Avena sativa L.—Continued						
var. *mutica*. (Awns vary in the different lots)	R 52g		3–	3	4–	3+
Do	R 53e		3	3	4	
Do	R 330a		4	4	4	4
Do	R 331a	1716	4–	4	4–	4–
var. Nebraska No. 21	R 280a	841	3+	4–	3–	3+
var. New Market	W 1048		3–	2+	4–	4–
var. *nigra*. (Closely resembles Black Mesdag)	R 70g		4	3	4	4–
var. North Finnish	R 123e	1882	4–	3+	4	3+
Do	C 1002	1801	3+	3+	3+	4
var. Palouse Wonder	W 748		3+	4–	4	4–
var. Potato. (Early Mountain)	W 761	1192	3+	3+	3–	4–
var. *praegraris*	R 62f	1773	4–	3		4–
Do	R 332a	1773	4–	4–	4	3
Do	R 333a	1775	3	3+	3	4–
var. President. (Closely resembles Swedish Select)	R 319a	1629	3–	4	4	4–
var. Richland	R 281a	787	0+	1–	1	1–
Do	R 320a	787	0+	1–	1–	1–
var. Roosevelt. (Closely resembles Swedish Select)	R 321a	1632	3	4–	4–	3
var. Rossman	R 322a	1688	3+	4	4–	3+
var. Ruakura	I 73	791		1–		1+
var. Sandwich. (Garton No. 5)	W 758	1194	3–	4–	3+	3
var. Scottish Chief	R 124e	1901	4–	3	2	4
var. Siberian	R 282a	741	4–	4–	3	4–
Do	R 323a	1712	3	4	4	3
var. Silvermine	R 220a	659	4	3+	4	4
Do	I 20			3		4
var. Sixty-Day	R 223a	165	4	3	2	2
Do	C 1004		3+	4–	3+	2+
Do	W 661	165	3+	3	3–	2+
var. Sixty-Day Selection (of Etheridge)	C 1010	1906	3+	3–	3+	3–
var. Snoma. (Received as Snoma Winter. Seed mixed, and identification of this resistant strain doubtful)	C 274		1	1	1–	1
var. Swedish Crown	C 1006		3	3–	4–	4
Do	W 1035		3	3+	4–	4–
var. Swedish Select	R 226a	134	4–	3–	4	4
Do	R 228a	552	4	4–	3	3
Do	R 231a	1634	3+	4–	3–	3+
Do	W 662		4–	3	3	3
Do. (Ligowa)	R 227a	1740	4–	3+	4–	4–
Do	W 1088	1740	3	3	3+	3
Do	I 59			4–		4–
var. Swedish Victory	C 1007		4–	4	4–	4
var. Tobolsk	R 122e	1893	3+	3	4	4
Do	R 232a	1709	3–	4–	4–	4
var. *trisperma*	R 334a	1776	3–	4	3	3+
var. Victor	R 126f	1875	4–	3	4	4–
Do. (Received as Black Tartarian)	C 1028		3–	4–	4	4–
Do. (Received as Black Alaska)	W 1023		3	3+	4	3+
var. Victory	W 780		3+	4–	3+	3+
Do	R 283	560	4–	4–	4–	4
var. Vogt New Era	C 1023		3	4–	3+	3+
var. Wernicke's Golden	R 324a	1672	4–	4–	4	3
var. White Bonansa	R 325a	1650	4	4	3+	3
var. White Maine	R 326a	1651	4–	4	4–	4–
var. White Queen	R 327a	1700	4	4	3	4
var. Winter Turf. (Lots of Winter Turf differ in kernel color and awns)	R 169a		4–	3+	3–	3
Do	R 235a	195–15	3–	2+	3+	4
Do	R 236a	274–20	3	2+	3–	4–
Do	R 237a	673	4–	4–	3	4
var. Winter Turf. (Received as Gray Winter)	W 1030		3–	3–	4	4
var. Black X Sixty-Day	W 1186		4–	3+	3	3+
Avena sativa orientalis L.						
var. Black Tartarian. (Received as Black Diamond)	R 308a	1763	4–	4	3+	4–
Do	W 762		3	3+	3+	3–
var. Eclipse. (Mixed, containing resistant strain of White Tartar type)	R 274a	843	3	3+ & 1–	4–	1– & 4

TABLE I.—*Result of inoculation of oat varieties with stem rust at Berkeley, Calif., in the summer of 1920, as measured on a scale of 0 to 4, in which 0 represents immunity and 4 represents complete susceptibility*—Continued

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
Avena sativa orientalis L.—Continued						
var. Eclipse	R 312a	843	4−	4	4	3+
var. Garton 748	R 298a	1612	4−	4−	4−	3
Do	R 109e	1863	4	4−	4	3
var. Garton Gray	R 172a		4−	3+	4	4−
var. German type	R 335a		4	4	4−	4−
Do	R 336a		4−	4	4	4
Do	R 337a		4	4	4	4−
var. Golden Giant	R 173a	1866	4	4−	3+	3+
var. Green Mountain (White Tartar type)	R 110e	1872	1	1	1	1−
var. Improved Roberts	C 1017		3+	4−	4−	4−
var. Long's White Tartar (White Tartar type)	C 1026		1+	1+	1+	1−
var. *mutica* (White Tartar type)	R 321		1	1	2−	1
var. *obtusata*	R 331		4−	4−	3−	3
var. *setosa*	R 302a	1618	4	4−	2	4−
var. Short Tartarian (Sparrowbill type)	C 1027		3+	4−	4	3−
var. Sparrowbill	R 111e	1867	4−	3	3+	4
Do	C 1030	1812	4−	4−	3+	3
var. Storm King	R 112d		3+	4−	4	4
Do	C 1001		3+	4	3+	4−
var. White Egyptian (Sparrowbill type)	R 300a	1605	2	4	4−	4
var. White Tartar. (Mostly received as White Russian)	R 284a	551	1−	1	0+	1+
Do. (Received as Improved Sonoma Winter)	C 1016		1+	1	1	1
Do	R 301a	1614	1−	1+	1	1
Do	I 98–4			1−		1−
Do	I 102½			1		1−
Do	I 102½–5		1+	1	2−	1−
Do	I 102½–7		1	1	1	0+
Do. (Received unnamed)	K 6096		1		1−	
Do	R 174a		1−	1	1−	1+
Do. (Received as Tartarian)	C 1020		1+	1+		1
Do. (Received unnamed; has more awns than normal)	R 299a	1615	3+	4−	4−	4−
Do	W 739	441	1	1		0+
Avena sterilis L.						
var. Bathurst No. 4	C 1014	1810	3	4−	3+	4−
var. Burt	R 67n		4	3+	2+	2+
Do	R 74k		4	4−	3	3
Do	R 175a		4−	4	2+	2+
Do	R 253a		4−	4	3	3+
Do	C 1005	1798	4−	4−	3−	3+
Do	I 25		3+	3+	3−	3−
var. Burt Selection	C 1011		3+	4	4−	3+
var. California Red	C 1003	1026	4−	4	3	4−
var. Early Ripe	R 75j		4−	3+	4	4
Do	I 26			3−		3−
var. Fulghum	R 257a	708	4−	4−	3	
Do. (Received as King)	R 129f	850	4−	3+	2+	2+
var. Improved California Red	C 1013	1811	4−	4	3−	3+
var. Italian Rustproof	R 258a	388–6	3	3+	3+	4−
Do	R 259a	388–6		3+		3
var. *ludoviciana* Dur.	R 291a	1781	3+	4	3+	4
var. *nigra*. (Received as Black Oat)	C 1008		4	4	4−	4
Do	R 130c		3	3+	3−	3
var. No. 356	C 1009		4	4	3−	3−
var. Red Algerian	C 286	286	3+	4	3+	3+
var. Red Rustproof	R 263a		4−	4−	3	3
Do	R 265a		4−	3+ to 4−	3	3+
Do	I 67			3		3−
var. Sterilis Selection (of Etheridge)	R 132e	1859	4−	4−	3−	3
var. Sunrise	C 1012	1799	3−	4−	3+	3+
var. Red Rustproof	W 768		3	3+	3	2+
var. Turkish Rustproof	R 267a	356	4−	4−	3+	3+
Do	R 268a	356–12	4−		3+	4
var. (Received unnamed)	R 28k		4−	4	3+	4
Avena strigosa Schreb.						
var.	R 29j		4−	4−	3+	2
var.	R 133e	1782	4−	4	3−	3−
var. (Received as *Avena barbata*)	I 119–3			2+		3
Do	I 119–4		3	3+	3−	3+

INVESTIGATIONS

Two distinct experiments were conducted. In the first a single rust, collected at Berkeley, Calif., was used. In the second, nine different collections of rust, from as many localities in the State, were used for inoculations.

EXPERIMENTS WITH ONE RUST IN GREENHOUSE AND FIELD

The varieties of oats tested were obtained from the following sources:

	Varieties
United States Department of Agriculture	140
California Agricultural Experiment Station	36
Washington Agricultural Experiment Station	26
Iowa Agricultural Experiment Station	14
Kansas Agricultural Experiment Station	1
Total	217

The lists included duplicates of many of the varieties. Some of the plants bearing the same name were very similar morphologically, but others differed somewhat. All were included in the tests. In other cases, plants bearing different names were alike. Etheridge's "Classification of the Varieties of Cultivated Oats" (*3*) was used in studying the material and any changes that were made in the names were in conformity to his descriptions. Each change in the name of a variety is recorded.

The oat stem rust used in 1920 to obtain the results shown in Table I was found growing on cultivated oats in the botanical garden at Berkeley in the winter of 1919–20. It was cultured on red oats in the greenhouse. Square paper pots were placed in flats holding four rows of pots, with 10 in each row. Each variety occupied one row in a flat, a single seed being sown in each of the 10 pots. As the available greenhouse space was not great enough to accommodate all of them at once, they were grown in successive lots. The seedlings were inoculated when they were about 10 days old and were kept in a damp chamber 48 hours. After two weeks the rust was studied and recorded, and the rusted leaves were packeted, labeled, and pressed. The seedlings were then transplanted to plats in the botanical garden. The first lots were set out on the seventeenth of February and the last on the twenty-seventh of March. The series was then repeated, the second set being about six weeks later than the first.

When half grown these plants were subjected to a heavy artificial epidemic of the same rust. For this purpose urediniospores were grown in the greenhouse. These spores were put into tap water and applied to the plants either by the use of an atomizer or by rubbing the culms with moistened fingers. As the epidemic developed on the older plants spores were transferred by the same method from the older to the younger plants. When nearing maturity, rusted specimens of each variety (including at least one complete culm) were studied, recorded, packeted, and pressed. Thus, each variety was subjected to four rust tests, two in the greenhouse and two in the field. The rust was measured on a scale of 0 to 4, in which 0 is immunity and 4 is complete susceptibility.

Eriksson and Henning (*2*), in "Die Getreideroste" (1896), used in their tables a scale of 1 to 4 for grading rust. Rust-free material was recorded in a column headed "rein," 1 indicated traces of rust, 2 and 3 larger amounts, and 4 abundance of rust. Their estimates were based largely on the quantity of rust found.

As used in this paper, the scale of 0 to 4 is not based on quantity of rust but on the size and appearance of individual uredinia. The types of infection as defined

by Stakman and Levine (*16, p. 5*) and their associates have been used in these studies. They are as follows:

0. IMMUNE.—No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues.

1. VERY RESISTANT.—Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive, necrotic areas.

2. MODERATELY RESISTANT.—Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green, but slightly chlorotic, islands.

3. MODERATELY SUSCEPTIBLE.—Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.

4. VERY SUSCEPTIBLE.—Uredinia large, numerous, and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

X. HETEROGENEOUS.—Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill defined.

Table I shows that the great majority of the varieties tested are susceptible to the oat stem rust used (usually grading from 3− to 4), a few are highly resistant (grading from 1− to 1+), and not one is fully immune (grade 0).

The oats fall naturally into two well-defined groups on the basis of rust resistance, and there are few intermediates. Some of the slender varieties with weak straw may not show as large uredinia or as abundant spore production as the stouter-stemmed varieties with greater substance, but they may be quite as susceptible. It is quite possible, too, that of two varieties showing equal susceptibility in the seedling tests in the greenhouse one may mature earlier than the other and so escape the worst ravages of the disease. This is quite distinct from the question of susceptibility.

Examples of the susceptible group are given in Plate 1. A is a specimen of *Avena sativa* L. (Silvermine, I 20) and B is *Avena sativa orientalis* L. (Short Tartarian, C 1027), a type resembling Sparrowbill. In both varieties the fungi make an abundant growth and produce well-developed uredinia often confluent into broad streaks several inches long and bearing urediniospores in abundance. In sharp contrast to this are the resistant forms. The specimens photographed (Pl. 2) belong to *Avena sativa orientalis* and are of the White Tartar (White Russian) type. A was listed as Long's White Tartar (C 1026) and B as White Tartar (C 1020). The fungus enters these hosts as readily as the susceptible varieties. In fact, each specimen is peppered with hundreds of minute uredinia, giving it a speckled appearance, but the development of each fungus is sharply limited, the sorus remains minute, and its spore output is negligible. Examination shows that it frequently contains teliospores.

Resistant varieties were found only in *Avena sativa* L. and its subspecies *A. sativa orientalis* L. All the available lots of *A. brevis* Roth., *A. fatua* L., *A. nuda* L., *A. sterilis* L., and *A. strigosa* Schreb. were found to be susceptible.

For convenience the data on the resistant varieties of *Avena sativa*, from Table I, are presented separately in Table II. The two lots of the variety Richland are similar. The plants are erect, with fine, short culms, narrow leaves, and small sparsely-branched panicles of the general type of Kherson. The glumes are short, the kernels light yellow and awnless, and there is an occasional three-kerneled spikelet. The reaction to rust is 0+ to 1, so far as observed.

Four lots of Green Russian are included in the varieties tested but only one lot proved uniformly resistant to stem rust. The first of these lots (see Table I) contained only susceptible plants; the second and third contained a small percentage of markedly resistant ones in addition to those susceptible; and the fourth, Iowa 91–4, appears to be a selection containing only the resistant strain.

In the subspecies *Avena sativa orientalis* L., 15 lots proved resistant. They were received from several sources under seven different names. Close examina-

tion of the plants shows that they differ only in minute morphological details. They are all of the White Tartar (White Russian) type. The data on these, from Table I, are given separately in Table III.

The seed of the variety called Eclipse was mixed and produced, in addition to the resistant plants, others which were markedly susceptible.

TABLE II.—*Data on varieties of Avena sativa resistant to oat stem rust*

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
Avena sativa L.:						
var. Green Russian	I 91-4	.....	.....	1−	.....	2−
var. Richland	R 281a	787	0+	1−	1	1−
Do	R 320a	787	0+	1−	1−	1−
var. Ruakura [a]	I 73	791	.....	1−	.....	1+
var. Snoma [b]	C 274	.....	1	1	1−	1

[a] The variety Ruakura has the same general habit as Richland but has much longer glumes. The kernel is whitish and the awns are few and delicate or wanting. The rust test runs from 1− to 1+.

[b] Snoma is a gray oat of the Winter Turf type, more or less spreading, with a narrow leaf, narrow stiff panicle, and a few geniculate awns.

TABLE III.—*Data on varieties of Avena sativa orientalis of the White Tartar (White Russian) type which are resistant to stem rust*

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
Avena sativa orientalis L.:						
var. Green Mountain	R 110e	1872	1	1	1	1−
var. Long's White Tartar	C 1026	.....	1+	1+	1+	1−
var. *mutica*	R 321	.....	1	1	2−	1
var. White Tartar [a]	R 284a	551	1−	1	0+	1+
Do	R 301a	1614	1−	1+	1	1
Do	I 98-4	.....	.....	1−	.....	1−
Do	I 102½	.....	.....	1	.....	1−
Do	I 102½-5	.....	1+	1	2−	1−
Do	I 102½-7	.....	1	1	1	0+
Do	K 6096	.....	1	.....	1−	.....
Do	C 1016	.....	1+	1	1	1
Do	R 174a	.....	1−	1	1−	1+
Do	C 1020	.....	1+	1+	.....	1
Do	W 739	441	1	1	.....	0+
var. Eclipse	R 274a	843	3	3+&1−	4−	1−&4

[a] White Tartar is the accepted name for White Russian.

EXPERIMENTS WITH NINE COLLECTIONS OF RUST

At the time when this work was done (1920) physiological races of oat stem rust had not been reported, but their existence seemed probable. Experiments were undertaken in the hope of determining whether there are one or several physiological races of oat stem rust in California, and whether the oat varieties resistant to the rust collected at Berkeley are resistant to stem rust collected throughout the State.

Specimens of oat stem rust were obtained from nine oat-growing districts in California. The shaded areas of figure 1 show the approximate distribution of oat-stem rust in California based on the collections of 1919, and the numbers mark the nine localities from which the collections cultured were obtained. Stock cultures of these were kept growing, isolated from each other in cheesecloth cages, in the greenhouse. About 40 varieties of oats were chosen, including

representatives of all available species of Avena, and all of the varieties that proved resistant to the oat stem rust collected in Berkeley.

The experiments were made on seedlings in the greenhouse. Nine pots of seedlings of each variety were grown, one for each collection of rust. All were inoculated on the same day, placed under bell jars in the cages for 48 hours, the bell jars then removed and the plants allowed to grow in the pots. At the end of two weeks the plants were studied and rust infection recorded, and the rusted leaves packeted and pressed. On completion of the first series the entire experi-

FIG. 1.—Map of California showing, by the shaded areas, the distribution of oat stem rust in California in 1919, and by numerals, the points where the nine collections of rust were made

ment was repeated. The second experiment was not completed until late in the fall of 1920, when the cooler weather and reduced light proved unfavorable for the development of the rust. These data, therefore, were discarded and this part of the work was repeated in the spring of 1921.

The results are given in Table IV and show a remarkable uniformity in the reaction of any variety of oats to the strains of rust from different localities. In general, the varieties in the first experiment that were susceptible to the rust collected at Berkeley proved equally susceptible to rust from the other sources, and the varieties resistant to the Berkeley rust proved resistant to the nine collections from other localities.

A possible exception to this statement is the reaction to the rust collected at Eureka. In general, the reaction to it was the same as to the rusts from other sources, but in the numerous lots included under White Tartar (White Russian), which is uniformly resistant to all the other rusts used (except for an occasional rogue in R 284a and I 102¼), an occasional plant proved susceptible to the Eureka rust. One or two plants susceptible to the Eureka rust were found in Green Mountain, R 110e; Long's White Tartar, C 1026; White Tartar (White Russian), I 102¼–7; and White Tartar (White Russian), C 1016. These occurrences are indicated by the footnote *c* in Table IV. It is possible that these susceptible plants also are rogues; but it seems unlikely, because the same lots of seed produced plants which reacted uniformly to the rust from other sources.

It was reported that in Eureka stem rust occurred on Richland, another resistant variety. Rust specimens were obtained from Eureka at three different times. In each case the material contained a mixture of crown and stem rusts, of which only the crown rust grew on the Richland. It may be that a form of stem rust occurs there to which Richland is susceptible, but, if so, the writers failed to obtain it. Our only indication of a different rust form is the reaction to White Tartar (White Russian), and this is inconclusive evidence.

Stakman and Levine and their associates have described in several of their papers (*16*, *18*) a condition in which the infections on a single leaf vary greatly in size and vigor. They have named this the heterogeneous or X type of infection as noted earlier in this paper.

The oat stem rust on Joanette (R 125e) showed a similar variability and may belong to the X type. Some plants of Joanette appeared to be susceptible, some resistant, and some showed two or more distinct reactions on the same leaf. No explanation of this condition can be offered at present with any assurance.

Stakman, Levine and Bailey (*17*, *18*) in their reports on specialization of *Puccinia graminis avenae* distinguished certainly four, and perhaps five, physiological races of stem rust. Victory (C. I. 1145), White Tartar (C. I. 1614), and Monarch Selection were used as differential hosts, with results which may be summarized roughly as follows:

Form No.	Victory	White Tartar (White Russian)	Monarch Selection
I	Susceptible	Resistant	Immune.
II	do	do	Susceptible.
III	do	Intermediate in resistance.	Do.
IV	do	Susceptible	Do.
V			Heterogeneous type.

So far as can be judged from the limited data available, all the collections of oat stem rust used in the experiments represent the physiological race II, as plants of Victory and Monarch Selection were susceptible and White Tartar was highly resistant.

TABLE IV.—*Comparative test of oat varieties in greenhouse at Berkeley, in 1920 and 1921, with stem rust from nine localities in California*

TABLE IV.—*Comparative test of oat varieties in greenhouse at Berkeley, in 1920 and 1921, with stem rust from nine localities in California*

Name	Lot No.	C. I. No.	Date inoculated	Rust collected at—								
				Balboa Park	Berkeley	Davis	Dodgeland	Eureka	Riverside	Salinas	Winchester	Woodland
Avena brevis Roth. var.	R 134d		Sept. 30, 1920	3−	3	4−	3−	3−	3−	3+	3−	3+
			May 19, 1921*	2	2+	2+	2	2+	2	3−	2	2
Avena fatua L. var.	R 290a	1779	Sept. 30, 1920	4−	4−	4−	3+	3	4−	4	3+	3−
			May 21, 1921	4	4−	4	3	3+	4−	4−	3+	3
Avena nuda L.												
var. *chinensis*	R 301		Sept. 27, 1920	4	4	4	4	4	4	4	4	
			May 31, 1921	4	4	4	4	4	4	4	4	4
var. *inermis*	R 294a	1768	Sept. 24, 1920	4	4	4	4	4	4	4	4	
			May 19, 1921*	3−	3−	3+	3+	3−	3+	4−	3−	3−
Avena sativa L.												
var. Abyssinian	R 303a	1747	Sept. 24, 1920	4	4	4	4	4	4	4−	3+	
			May 19, 1921*	3−	3+	3−	3+	3+	3	3+	3−	3−
var. Black Mesdag	R 117e	1877	Oct. 8, 1920	4−	4−	4	3+	4	4	4	4−	3+
			May 13, 1921	4	3+	3+	4−	3	4−	4−	4	3
var. Black. (Received as *Avena sterilis*).	R 295a	691	Oct. 4, 1920	4−	3+	4−	3+	3+	3	3+	3	3
			May 17, 1921	3	3	3+	3+	3	3	3+	4−	3
var. C. I. 602 (of Etheridge)	R 145a	602	Nov. 24, 1920*	2+	3−	3−	3−	3+	2+	3	3+	4−
			May 13, 1921	4−	4−	4−	4	4	4	4	4	3+
var. Culberson	R 192a	273	Oct. 8, 1920	3+	3+	4	4−	3+	4−	4−	3	4−
			May 13, 1921	4−	3	4−	4	4	4−	3+	3+	4−
var. Garton 473. (Received as *Avena sativa orientalis*).	R 297a	1613	Nov. 24, 1920*	3−	2+		3	3	2+	3−	3	3+
			May 13, 1921	4−	4	4−	4	4	4−	4−	4−	3+
var. Green Russian. (Contains a resistant strain).	R 276a	827	Sept. 20, 1920	3+	4− and 1	4 and 1	3+ and 1−	4−	4	4	3	
			May 24, 1921	4− and 1+	4−	3+ and 1	4	3+ and 1−	3+	3+	4−	3+ and 1−
var. Green Russian	I 91–4		Sept. 17, 1920	1	1	1	1	1	1	1	1	
			Oct. 29, 1920*	1	1	1	1	1	1	1	1	1
var. Hay	R 317a	1622	Nov. 24, 1920*	2+	3	3−	2−	3−	2+	2	3−	3−
			May 17, 1921*	3−	3−	3	3	2+	3	3−	3+	3−
var. Joanette. (Infection may be heterogeneous or X type).	R 125e	1880	Oct. 12, 1920	3+ and 1	2+ and 1−	3 and 1	1−	3+ and 1	3 and 1	3 and 1	1−	1
			May 21, 1921	3+ and 1−	3 and 1	4 and 1−	4 and 1	3 and 1	4 and 1−	0 to 1	1− and 4	3+
var. Kherson	C 1021		Oct. 4, 1920	4	4	4−	4	4	4−	3+	3+	3+
			May 9, 1921	4−	4−	4	4	4	3+	3+	4	4
var. Kherson	W 1047	1198	Oct. 9, 1920	4−	3+	4	3+	4−	3+	3+	3	4−
			May 11, 1921	4	4−	4	4	4	4	4−	4	3+
var. Kherson (Wisconsin Pedigree No. 156).	C 1029		Oct. 4, 1920	4	3	4	3	4−	4	4−	3	2+
			May 9, 1921	4	3	4	4	4	4	4	4	4
var. Richland	R 281a	787	Sept. 10, 1920	1−	1	1−	1−	1	1−	1−	1−	
			Oct. 23, 1920	0+	0+	1−	1−	1−	1−	1−	0+	0+
var. Richland	R 320a	787	Sept. 20, 1920	1−	1	1−	1−	1−	1−	1−	0+	
			Nov. 5, 1920	0+	0+	1−	1−	1−	1−	0+	1	0+

* The plants inoculated on these dates were affected by cold, cloudy weather, which reduced the size of the uredinia.

TABLE IV.—*Comparative test of oat varieties in greenhouse at Berkeley, in 1920 and 1921, with stem rust from nine localities in California*—Con.

Name	Lot No.	C. I. No.	Date inoculated	Rust collected at—								
				Balboa Park	Berkeley	Davis	Dodgeland	Eureka	Riverside	Salinas	Winchester	Woodland
Avena sativa L.—Continued												
var. Ruakura. (An occasional susceptible plant, probably a rogue.)	I 73	791	Nov. 16, 1920	1	1+	1−	1−	1+	1	1−	0+	1−
			May 11, 1921	1	1−	1	1−	1	1	1	1−	1−
var. Sixty-Day Selection (of Etheridge). (Closely resembles Burt.)	C 1010	1906	Oct. 13, 1920	3−	3+	4	4	4−	4−	4−	3−	3
			May 9, 1921	4	4	4	4	4	4	3+	4	4
var. Snoma. (Received as Snoma Winter. Seed mixed, one strain susceptible, the other resistant.)	C 274	------	Sept. 20, 1920	3+ and 1	3 and 1	4 and 1+	3+ and 1+	1+	4− and 1	4− and 1	3+ and 1	------
			May 24, 1921[b]	1	1+	1+	1+	1	1	1+	1	1
var. Winter Turf	R 237a	673	Oct. 13, 1920	3−	3	4	3	3	3+	3+	2+	3
			May 21, 1921	3+	4	4	4	4	4−	4	4	4
Avena sativa orientalis L.												
var. Eclipse. (Contains a resistant strain.)	R 274a	843	Sept. 10, 1920	3+ and 1	3+	4−	4−	4 and 1	4	4 and 1	3+	------
			May 24, 1921	3+	3+	3+	4	3+	3+	3 and 1	3	3
var. Green Mountain. (White Tartar type.)	R 110e	1872	Sept. 14, 1920	1−	1	1+	1+	1	1−	1+	1+	------
			Oct. 27, 1920	1	1+	1−	1	1[a]	1	1	1	1−
var. Long's White Tartar	C 1026	------	Sept. 22, 1920	1−	1	2−	2−	1	2−	2−	1	------
			May 11, 1921	1	1	1	1+	1+[c]	1	1	1	1+
var. *mutica* (White Tartar type).	R 321	------	Sept. 10, 1920	1	1+	1+	2−	2−	1	1	1	------
			Nov. 16, 1920	1−	1−	1−	1−	1	1−	1−	1−	1−
var. White Tartar	W 739	441	Sept. 17, 1920	1+	1+	1	1+	1+	1	1	1+	------
			Nov. 5, 1920	1−	1−	1−	1−	1	1−	1−	1−	1−
Do. (Occasional susceptible plant.)	R 284a	551	Sept. 14, 1920	1+	2−	1	2−	1[c]	2−	1+	1	------
			May 21, 1921	1+	1	1	2−	2[c]	1	1		2−
Do.	R 301a	1614	Sept. 14, 1920	2−	2−	1+	2−	1	1+	2−	------	------
			Oct. 27, 1920	1	1	1	1	1	1	1	1	1
Do.	I 98–4	------	Sept. 22, 1920	1+	1	1+	1+	1+	1	1	1	------
			Nov. 5, 1920	1	1−	1−	1−	1−	1−	1−	1−	1−
Do. (Occasional susceptible plant.)	I 102½	------	Sept. 20, 1920	1−	1	1−	1	1+	1	1	1−	------
			Nov. 2, 1920	1	1−	1−	1−	1−	1−	1	1−	1−
Do.	I 102½–5	------	Sept. 22, 1920	1	2−	2−	1+	2−	2+	1	1−	------
			Nov. 8, 1920	1−	1	1	1	1	1	1	1−	1−
Do.	I 102½–7	------	Sept. 22, 1920	1+	1+	2−	1+[c]	1+[c]	1	1+	1	------
			Nov. 8, 1920	1	1−	1−	1−	1−	1−	1−	1−	1−
Do. (Received unnamed.)	K 6006	------	Oct. 29, 1920	1−	1−	1+	1−	1	1	1	1−	1−
Do. (Received as Improved Snoma Winter.)	C 1016	------	Sept. 17, 1920	1	1	1+	1	1[c]	1	1	1+	------
			May 9, 1921	1	1	1+	1	1	1	1+	1	1+
Do.	R 174a	------	Sept. 14, 1920	1	1+	2−	1	1+	1+	1+	1	------
			Oct. 27, 1920	1	1	1	1	1	1	1	1	1
				1	1	1	1+	1	1	1	1	1

| | | |

SUMMARY

Of 217 varieties of oats grown in the greenhouse and in the field, in 1920, after being inoculated with oat stem rust collected at Berkeley, Calif., the following were found to be resistant: Richland, Ruakura I 73, Snoma, one lot of Green Russian, and all lots of White Tartar (White Russian). Eclipse and two lots of Green Russian contained a mixture of resistant and susceptible plants.

Collections of oat stem rust were made from nine oat-growing districts in California in 1920 and about 40 varieties of oats, including all previously found to be resistant, were inoculated in the greenhouse with these rusts in 1920 and again in 1921. In general, the resistant varieties proved resistant to the rust from all of these sources, and the susceptible varieties were susceptible to all.

A possible exception to this rule is the reaction of White Tartar (White Russian) to the rust from Eureka. In each of four lots of White Tartar there were one or two plants susceptible to the rust from Eureka. The same lots of White Tartar were uniformly resistant to the rusts from other parts of the State.

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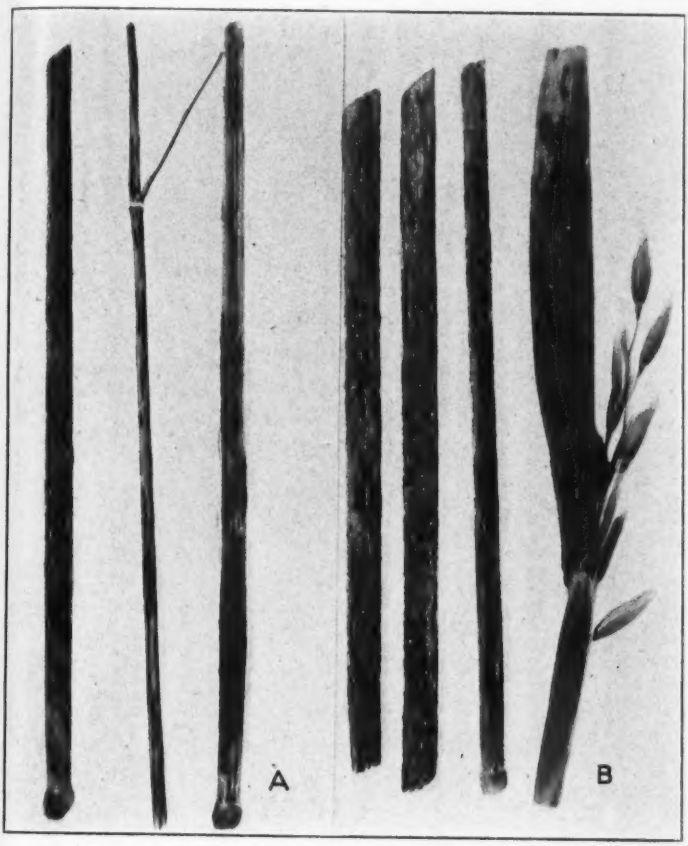
PLATE 1

Susceptible type

A.—Oat stem rust on Silvermine oat, I 20. Uredinia large and confluent.
B.—Oat stem rust on Short Tartarian oat, C 1027.


A
B

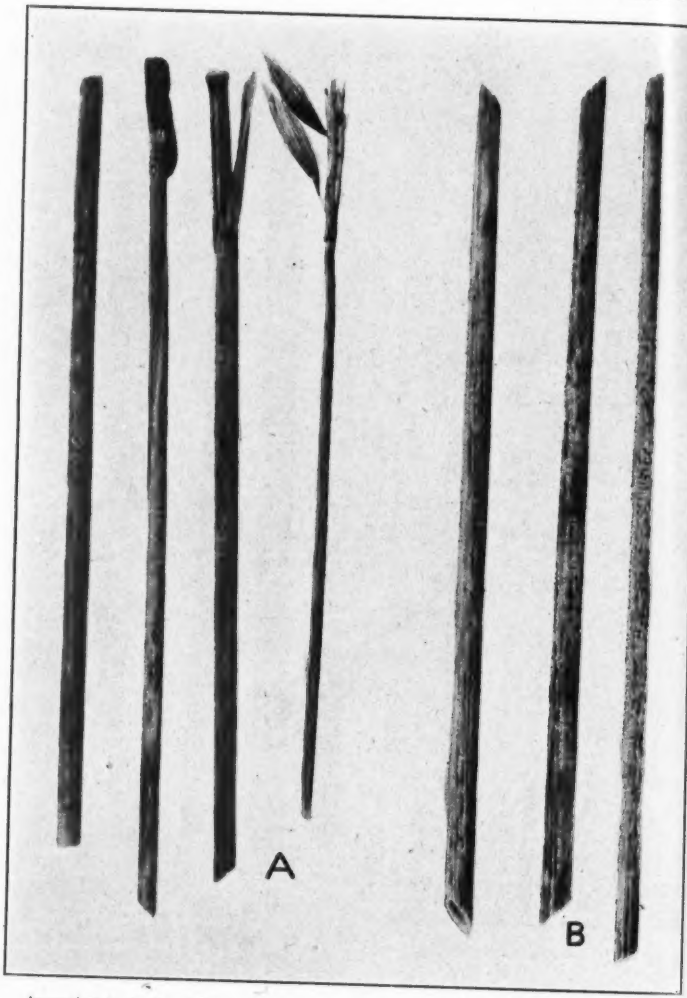

A
B

PLATE 2

Resistant type

A.—Oat stem rust on Long's White Tartar, C 1026. Sori numerous but minute. Host but little harmed.

B.—Similar infections on White Tartar, C 1020.